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3M

May 18, 2000

MR 36136

VIA EXPRESS MAIL

Dr. Charles Auer
Director
Chemical Control Division
Office Of Pollution Prevention And Toxics
United States Environmental Protection Agency
401 M Street, Southwest
Room 403 East Tower (Mail Code 7405)
Washington, D. C. 20460

CONTAINS NO CBI

Re: **Information On Perfluorooctane Sulfonates And Related Compounds**

Dear Charlie:

Pursuant to our recent communications, 3M is enclosing additional information on perfluorooctane sulfonates and related compounds. The enclosed information supplements information submitted to you previously under cover of our April 21, 2000 and May 4, 2000 letters. Again, we are providing this information on a voluntary basis as part of our continuing discussions with EPA regarding fluorochemistry. Please note that some of this information qualifies as confidential business information (CBI); we also have enclosed a sanitized version of CBI documents.

The enclosed information covers perfluorooctane sulfonates and certain related compounds listed in Table 1 of the document entitled: "Sulfonated Perfluorochemicals In The Environment: Sources, Dispersion, Fate And Effects", at 12 (March 1, 2000). Specifically, the information covers the following chemicals:

⇒ Perfluorooctane sulfonates, including CAS numbers 1763-23-1 (acid); 29081-56-9 (ammonium salt); 70225-14-8 (DEA salt); 2795-39-3 (potassium-salt); 29457-72-5 (lithium salt).

⇒ Perfluorooctanesulfonyl fluoride

85000000010

⇒ Perfluorooctanesulfonamide

⇒ Perfluorooctane sulfonylamido (ethyl) acetate

⇒ Perfluorodecanesulfonate

⇒ Perfluorohexane sulfonate

⇒ N-ethyl perfluorooctanesulfonamide

⇒ N-methyl perfluorooctanesulfonamide

⇒ N-ethylperfluorooctane sulfonamidoethanol

⇒ N-methylperfluorooctane sulfonamidoethanol

⇒ N-ethylperfluorooctanesulfonamidoethyl acrylate

⇒ N-ethylperfluorooctanesulfonamidoethyl acrylate

⇒ N-ethylperfluorooctanesulfonamidoethyl methacrylate

⇒ N-methyl perfluorooctanesulfonamidoethyl acrylate

3M has provided you with information on perfluorooctane sulfonates previously under cover of our April 21, 2000 and May 4, 2000 letters. We are enclosing some additional information on perfluorooctane sulfonates located as part of our continuing file search. For each of the foregoing related compounds, the enclosed information includes the following:

⇒ Copies of post-1976 studies and certain other information relating to the following environmental science areas: (i) physical and chemical properties; (ii) environmental fate and transport; (iii) environmental monitoring; and (iv) ecotoxicity. For each study, 3M has prepared a summary in the HPV "robust summary" format. 3M already has provided you with a general executive summary addressing each of these areas under cover of our May 4, 2000 letter.

⇒ Copies of post-1976 studies and certain other information relating to the following health effects areas: (i) acute toxicity; (ii) genotoxicity; (iii) repeated-dose toxicity; (iv) pharmacokinetics; (v) teratology; and (vi) medical surveillance and epidemiology. 3M has included a detailed index of this information subdivided by each compound.

⇒ Lists of all studies in progress and planned studies, along with study protocols or study plans, where available.

⇒ A bibliography of pre-1976 studies subdivided by each compound.

⇒ A bibliography of acute toxicity studies subdivided by each compound, except that we are providing copies of key acute studies (with reference to the HPV guidance).

⇒ A bibliography of published studies in 3M's possession.

⇒ An index of studies in 3M's possession believed to be in the FIFRA docket. Please note that this index has been submitted as CBI. 3M already has provided the Agency with a list of all fluorochemical-related submissions made by 3M to the TSCA Section 8(e) docket.

3M is continuing its file review and will supplement the enclosed information as appropriate. As you review this information, we ask that you bear several points in mind:

⇒ The enclosed information spans several boxes. We have organized the information in each box with labeled file folders and indices to aid EPA's review. To ensure that you and your staff are able to access the most pertinent information, we also are attaching to this letter the indices covering studies and other information.

⇒ Many of the studies refer to the test substance only by a 3M product identification number with a "T", "L" or "FC" prefix. Although analysis of the test substance has been included, where available, it is often not possible to determine from the study report itself the identity of the test substance. Thus, we have used the index to provide this information based on 3M's historical records keyed to the 3M product number to determine the composition of the test substance and have provided specific information on the index, such as percent composition, solvent context, salt form and purity grade. Please note that the term on the index "wide range" refers to a lower purity grade of the compound, and "narrow range" refers to a higher purity grade. Finally, it should be recognized that product formulations have evolved over the years, and thus, some of the test substances do not constitute current products.

⇒ We have included some additional studies on perfluorooctane sulfonates located as part of our continuing file review. As we have discussed, we plan to provide you with information on mixtures containing perfluorooctane sulfonates in the near future. Please be advised, however, that we already have provided you with studies and other information on aqueous and/or solvent solutions of

perfluorooctane sulfonates. As to the foregoing related compounds, the enclosed information likewise includes studies and certain other documents on both the compounds as well as on aqueous and/or solvent solutions of these compounds. We are not providing to you today, however, information on other types of mixtures containing these compounds and would like to discuss such information with you.

⇒ Consistent with the information previously provided on perfluorooctane sulfonates, 3M has not provided you with all analytical chemistry reports on these related compounds. Rather, we have enclosed certain analytical chemistry reports which may prove useful to EPA in interpreting certain studies; understanding the details of analytical chemistry methods; or verifying human and biomonitoring data.

⇒ We provided previously under cover of our April 28, 2000 letter to Oscar Hernandez a "Use And Exposure Information Profile" or "UEIP" for perfluorooctane sulfonates. Our present intention is to provide you with completed UEIP forms for these related compounds early next week. 3M needs additional time to clarify industrial hygiene and other release information to ensure that the data is accurate and placed in appropriate context.

⇒ The information on perfluorooctane sulfonates sent under cover of our May 4, 2000 letter included documents relating to ongoing mechanistic research by Dr. Ken Wallace of the University of Minnesota. Please note that this research also encompasses these related compounds. In addition, Dr. Marion Anders of the University of Rochester Medical Center is performing comparative metabolism research relating to perfluorooctane sulfonates and these related chemicals; there are not documents yet available relating to this research.

3M looks forward to discussing the enclosed information with you and other EPA staff. In the meantime, please do not hesitate to contact me with any questions.

Very truly yours

William A. Weppner

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Enclosures

N-EtFOSE Alcohol Bibliography

Synonyms: FM 3422, FM 3923, FM 4635, FM 4120, F 9353, FC-10

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Water solubility	3M Env. Lab	11/15/77	Robust summary, relevant pages from "Analytical Methodology on FM 3422"
Letter Report. Boiling Point Information	AScI	11/30/98	Robust summary, letter report
Determination of Vapor Pressure Curve by Dynamic Method for U1463 (Et FOSE)	AScI	11/6/98	Robust summary, final report
Internal Memo - Melting Point of FM-3923 N-Ethyl FOSE Alcohol	3M SMMD Analytical Lab	11/22/99	Robust summary, letter report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Tech Report - Biodegradation Studies of Fluorocarbons, Request for Laboratory work - BOD/COD, Memo - Chronological Review of Biodegradation Studies on FM3422, Tech Report - Biodegradation Studies of Fluorocarbons - II. (Robust summary for 4 documents)	3M Env. Lab	8/12/76, July, 1977, 8/12/77, 1/9/78	Robust summary, 2 technical reports, work request, review document
Tech Report - Bioconcentration of FM3422 in Bluegill Sunfish and in Channel Catfish, Tech Report - Aquatic Fate of a Fluorochemical: FM 3422	3M Env. Lab	5/17/1977, 10/14/77, 3/8/93	Robust summary, 2 technical reports, critique by J.W. Gillett (Cornel)
Tech Notebook copies - Analytical from fish bioconcentration studies	G. Vraspir	1/15/76 - 2/1/78	???
Alkaline hydrolysis	3M Env. Lab	11/15/77	Robust summary, relevant pages from "Analytical Methodology on FM 3422"
Pilot studies on soil adsorption	3M Env. Lab	10,13,78, 5/19/93, 12/30/75, 3/7/77, 4/12/77, 5/9/77	Robust summary, page from technical notebook, critique from S. Boyd (MSU) and 2 pages of data summaries.
Evaluation of the Bioconcentration Potential of FM 3422	3M Env. Lab	8/16/78	Robust summary, critique from G.W. Gillett (Cornell), technical report
Tech Report - Adsorption of FM-3422 on Soil	3M Env. Lab	9/1/78	Robust summary, critique from S. Boyd (MSU), technical report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Tech Report - Analysis for Fluorochemicals in Bluegill Fish	3M Env. Lab	5/1/79	Robust summary, critique from J.W. Gillett (Cornell), technical report
Tech Report - Bioaccumulation of Fluorochemicals in Tenn. River, Tech Report - Fluorochemicals in Tenneessee River Fish.	3M Env. Lab	5/22/79, 12/28/79, 3/8/93	Robust summary, 2 technical reports, ciritque from J.W. Gillett (Cornell)
Technical Report - Photolysis of FM 3422 on Soil	3M Env. Lab	12/10/79	Robust summary, technical report, critique from S. Boyd, MSU
Tech Report - FM-3422 Photolysis Study using Simulated Sunlight	3M Ag. Products Lab	8/11/81	Robust summary, technical report

Summary Reports

Title	Laboratory or Author	Completion Date	Type
Tech Report - Analytical Methodology on FM 3422	3M Env. Lab	11/15/77	Technical report., Critique by S. Boyd (MSU) Exerpts from this report appended to solubility and alkaline hydrolysis robust summaries.
Tech Report - Analytical Methodology and Support	3M Env. Lab	1/17/79	Technical report. Exerpts from this report appended to solubility, alkaline hydrolysis,volatility, distribution coefficient, soil adsorption, biodegradation, and fish chronic toxicity robust summaries.

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
The Effects of Continuous Aqueous Exposure to 78.01 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (Pimephales promelas), Summary of Histopathological Examinations of Fathead Minnow (Pimephales promelas) Exposed to 78.01 for 30 Days..	EG&G Bionomics	June, 1978, Sept. 1978	Robust summary, 2 technical reports, exerpt from Tech Report - Analytical Methodology and Support (1/17/79)
Tech Report - Multi-Phase Algal Assay Test Method.	3M Env. Lab	12/30/81	Robust summary, technical report.

N-MeFOSE Alcohol

Synonyms: FM 3925, L4528, L-4309, PPA-791, PPA-790 (Me &Et FOSE mix) cc 796-10, FM 3974, cc 796-3

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Tech Report - Solubility of FM 3925	3M Env. Lab	1/8/79, 1/17/79	Robust summary, tech report, excerpt from Tech report - Analytical Methodology and Support, 1/17/79

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Tech Report - Biodegradation Studies on FM 3925, Tech Report - Chemical Oxygen Demand of FM 3925	3M Env Lab	1/8/79, 1/8/79	Robust summary,2 technical reports
Tech Report - Distribution coefficient of FM 3925 in n-Octanol/Water	3M Env. Lab	1/10/79	Robust summary, technical report
Tech Report - Adsorption of FM-3925 on Soil	3M Env. Lab	3/22/79, 5/19/93	Robust summary, critique from S. Boyd (MSU), technical report
Tech Report - Bioaccumulation of Fluorochemicals in Tenn. River Fish, Tech. Report - Fluorochemicals in Tennessee River Fish, Ar No. 7238 - Determination of Fluorinated Alcohols in Fish Extracts	3M Env. Lab	5/22/79, 12/28/79, 10/23/79	Robust summary, critique from J.W. Gillett (Cornell), 3 technical reports

Summary Reports

Title	Laboratory or Author	Completion Date	Type
None			

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Tech Report - Aquatic Toxicity Studies: FM 3925	3M Env. Lab	1/8/79	Robust summaries for fish and daphnia, copies of data sheets, technical report

N-Ethylperfluorooctanesulfonamide = Ethyl Amide

Synonyms:EtFOSA, EtPOSA, U1464, FX-12, F6309

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Determination of Vapor Pressure curve by dynamic Method for U1464 (ET POSA)	ASci Corporation	11/6/98	Robust summary, final report
Letter Report. Boiling Point Information	ASci Corporation	11/30/98	Robust summary, letter report

Environmental Fate and Transport

Title	Laboratory or Author	Date Completed	Type
Technical Report - Bioaccumulation of Fluorochemicals in Tenn. River Fish, Technical. Report - Fluorochemicals in Tennessee River Fish	3M Env. Lab	5/22/79, 12/28/79	Robust summary, 2 technical reports, 1 critique from J.W. Gillett of Cornel (3/8/93)
Environmental Laboratory Final Report for BOD/COD	3M Env. Lab	6/12/84	Robust summary, copy of lab report

Ecotoxicity Elements

Title	Laboratory or Author	Date Completed	Type
Fathead minnow static acute toxicity test - FX-12	3M Env. Lab	4/6/84	Robust summary, data sheets
Daphnia magna static acute toxicity test - FX-12	3M Env. Lab	4/8/84	Robust summary, data sheets
Acute Toxicity of U1464 to *Ceriodaphnia dubia*	ASci Corporation	5/4/98	Robust summary, final report
Aucte Toxicity of U1464 to Larval Fathead Minnow (*Pimephales promelas*)	ASci Corporation	5/4/98	Robust summary, final report
Inhibition of U1464 for Activated Sludge Respiration Inhibition	ASci Corporation	5/15/98	Robust summary, final report
Acute Toxicity of U1464 to *Daphnia magna*	ASci Corporation	5/20/98	Robust summary, final report

POSF

Synonyms: FX-8

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Water solubility estimate (screen)	3M Env. Lab	5/11/84, 3/1/93	Robust summary, critique by E. Tucker, lab worksheets

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Environmental Aspects of POSF	3M Env. Lab	9/1/83	Technical report
Reappraisal of POSF Environmental Fate	3M Env. Lab	12/15/83	Technical report

Summary Reports

Title	Laboratory or Author	Completion Date	Type
None			

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
96-hour Toxicity to Fathead Minnow	3M Env. Lab	4/6/84	Robust summary, data sheets
7-Minute Exposure Activated Sludge Toxicity Test	3M Env. Lab	5/10/84	Robust summary, data sheets

Methyl FOSEA

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Study of Stability of MeFOSEA in Aqueous Buffers Using Gas Chromatography with Atomic Emission Detection	3M Env. Lab	6/14/99	Robust Summary and Technical Report

Environmental Fate and Transport

Title	Laboratory or Author	Date Completed	Type
Determination of the Partition Coefficient (N-Octanol/Water) of T-5969.4	3M Env. Lab	11/9/94	Robust Summary and Technical Report

Ethyl FOSEA

Synonyms: FX-13, B1228, D-1

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
Determination of Physico-chemical Properties of Sample D-1	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	2/14/96	Robust summary, final report

Environmental Fate and Transport

Title	Laboratory or Author	Completion Date	Type
Ready biodegradability of FX-13 (BOD/COD)	3M Env. Lab	4/26/84	Robust summary, copies of data sheets
Ready Biodegradability Test of D-1	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	10/31/95	Robust summary, final report
Bioaccumulation Study of Sample D-1 with Carp (Cyprinus carpio)	Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan	11/30/95	Robust summary, final report

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Acute toxicity of FX-13 to fathead minnow	3M Env. Lab	4/1/84	Robust summary, copies of data sheets.

Perfluorodecanesulfonate, Ammonium Salt (PFDS)

Synonyms: FC-120, FC-121, LR J2904

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type
None Available			

Environmental Fate and Transport

Title	Laboratory or Author	Date Completed	Type
BOD/COD Testing Results FC-120	Pace Incorporated, Minneapolis, MN	12/4/91	Robust summary, lab report
BOD/COD Testing Results FC-121-X	Pace Laboratories, Inc, Minneapolis, MN	11/3/87	Robust summary, lab report

Summary Reports

Title	Laboratory or Author	Date Completed	Type
None available			

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Toxicity of FC-121-X to Microtox	3M Env. Lab	1987	Robust summary, test summary sheet
Toxicity of FC-121-X to activated sludge	3M Env. Lab	10/30/87	Robust summary, test summary sheet
Acute Toxicity of E2566-1 to Daphnia magna (FC-121-X)	ABC Laboratories, Columbia, MO	1/9/88	Robust summary, final report
Acute Toxicity of E2566-1 to Fathead Minnow (Pimephales promelas)	ABC Laboratories, Columbia, MO	1/15/88	Robust summary, exerpts from final report
Static Acute Toxicity of FC-120 to the Fathead Minnow, *Pimephales promelas*	EnviroSystems Division, Resource Analysts, Inc. Hampton, NH	March, 1992	Robust summary, final report

Static Acute Toxicity of FC-120 to the Daphnid, *Daphnia magna*	EnviroSystems Division, Resource Analysts, Inc. Hampton, NH	Feb., 1992	Robust summary, final report
OECD Activated Sludge Respiration Inhibition Test #209 - Toxicity of FC-120	3M Env. Lab	3/9/92	Robust summary, data sheets
Microbics' Microtox Test - FC-120	3M Env. Lab	4/20/92	Robust summary, data sheets

Perfluorooctane sulfonylamido(ethyl)acetate = PFOSAA

Synonyms: FC-109, FC-109-X, FC-128, FC-129, E2113, E2566-2, R1904

Physical/Chemical Properties

Title	Laboratory or Author	Date Completed	Type

Environmental Fate and Transport

Title	Laboratory or Author	Date Completed	Type
Tech. Report - Fate of Fluorochemicals in the Environmental (sic) (Warburg respirometer method for biodegradation of FC-128)	3M Env. Lab	8/12/76	Robust summary, technical report
Ready Biodegradability of FC-128 - BOD/COD	3M Env. Lab	7/8/77	Robust summary, copies of data sheets
Tech report - Analysis for Fluorochemicals in Bluegill Fish	3M Env. Lab	5/1/79	Robust summary, technical report
Ready biodegradability of FC-127 - BOD/COD	3M Env. Lab	8/19/81	Robust summary, copies of data sheets
Ready biodegradability of FC-109-X - BOD/COD	Pace Laboratories, Minneapolis, MN	12/31/87	Robust summary, copy of computer results from archives

Summary Reports

Title	Laboratory or Author	Date Completed	Type

Ecotoxicity Elements

Title	Laboratory or Author	Completion Date	Type
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	5/18/74	Robust summary, copies of data sheets
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	6/29/74	Robust summary, copies of data sheets
Acute toxicity of FC-128 to fathead minnow	3M Env. Lab	8/26/77	Robust summary, copies of data sheets
Acute toxicity of FC-127 to fathead minnow	3M Env. Lab	7/24/81	Robust summary, copies of data sheets
Toxicity of FC-127 to activated sludge respiration	3M Env. Lab	8/14/81	Robust summary, copies of data sheets

Ready Biodegradability: "Modified OECD Screening Test" for Fluorad FC-129	RCC, Itingen, Switzerland	9/19/84	Robust summary, memo about study reliability, final report
Microbics Microtox Toxicity Test - FC-109-X	3M Env. Lab	10/15/87	Robust summary, copies of data sheets
Activated Sludge Respiration Inhibition - FC-109-X	3M Env. Lab	10/30/87	Robust summary, copies of data sheets
Acute Toxicity of E2566-2 to Daphnia magna	ABC Laboratory, Columbia, MO	1/9/88	Robust summary, final report
Acute Toxicity of E2566-2 to Fathead Minnow (*Pimephales promelas*)	ABC Laboratory, Columbia, MO	1/12/88	Robust summary, final report
Biodegradation (methylene blue active substance)	Pace Laboratories, Inc., Minneapolis, MN	2/15/89	Robust summary, computer report, copies of data sheets
Acute Toxicity of R1904 to *Daphnia magna*	AScI Corporation, Duluth, MN	6/17/97	Robust summary, final report
Growth Inhibition of R1904 for Green Alga (*Selenastrum capricornutum*)	AScI Corporation, Duluth, MN	6/18/97	Robust summary, final report
Acute Toxicity of R1904 to Fathead Minnow (*Pimephales promelas*)	AScI Corporation, Duluth, MN	6/27/97	Robust summary, final report
Microbics Microtox Toxicity Test - FC-129	AScI Corporation, Duluth, MN	7/9/97	Robust summary, copies of data sheets
Inhibition of R1904 for Activated Sludge Respiration	AScI Corporation, Duluth, MN	7/10/97	Robust summary, final report
R1904 BOD Report for 3M Company	AScI Corporation, Duluth, MN	Jul-97	Robust summary, final report

Expected Completion Dates for Degradation and Transport Studies in Progress

Photolysis

Chemistry	buffer/H_2O_2	humic material	soils	Fe_2O_3	TiO_2
POSF	October, 2000	October, 2000	October, 2000	October, 2000	October, 2000
FOSA	August, 2000	August, 2000	August, 2000	August, 2000	August, 2000
N-EtFOSA	August, 2000	August, 2000	Not applicable	Not applicable	Not applicable
N-MeFOSA	December, 2000	December, 2000	Not applicable	Not applicable	Not applicable
N-EtFOSE Alcohol	July, 2000	July, 2000	July, 2000	July, 2000	July, 2000
N-MeFOSE Alcohol	February, 2001	February, 2001	Not applicable	Not applicable	Not applicable
N-EtFOSEA	September, 2000	September, 2000	Not applicable	Not applicable	Not applicable

Hydrolysis

Chemistry	buffers	soils
POSF	December, 2000	Not applicable
FOSA	August, 2000	Not applicable
N-EtFOSA	August, 2000	Not applicable
N-MeFOSA	September, 2000	Not applicable
N-EtFOSE Alcohol	July, 2000	July, 2000
N-MeFOSE Alcohol	September, 2000	Not applicable
N-EtFOSEA	September, 2000	Not applicable

Adsorption/Desorption

Chemistry	
POSF	November, 2000
FOSA	January, 2001
N-EtFOSA	January, 2001
N-MeFOSA	March, 2001
N-EtFOSE Alcohol	September, 2000
N-MeFOSE Alcohol	March, 2001
N-EtFOSEA	March, 2001

Atmospheric Photolysis

Chemistry	
POSF	August, 2000
FOSA	Not applicable
N-EtFOSA	Not applicable
N-MeFOSA	Not applicable
N-EtFOSE Alcohol	September, 2000
N-MeFOSE Alcohol	Not applicable
N-EtFOSEA	Not applicable

All studies underway at the 3M Env. lab

Laboratory Environmental Studies in Planned and in Progress

Chemistry	Study	Conducted By	Expected Completion Date
	Physical/Chemical Properties		
N-EtFOSE Alcohol	Solubility in Water - In progress	Wildlife International, Easton, MD	Sept., 2000
	Degradation and Transport		
	See next page		
	Ecotoxicology		
	None planned		

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

1. POSF	**Perfluorooctanesulfonyl fluoride**

Acute Toxicity

1) Primary Skin Irritation Test with T-3874 in Albino Rabbits, Pathology and Toxicology, Riker Laboratories, Project No. 0386EB0135, 3M Reference No. T-3874, April 14, 1986

2) T-3607 Acute Inhalation Toxicity Test, Bushy Run Research Center, Project No. 47-527, 3M Reference No. T-3607, December 19, 1984

3) Acute Oral Toxicity Study – Method, Summary, Pathology and Raw Data Appendix, Hazleton Laboratories America, Inc., Project No. 40703983, October 5, 1984

Genotoxicity

1) In Vitro Microbiological Assays of 3M Company Compounds T-2540 CoC and T-2541 CoC, SRI International, Project No. LSC 4442-16, 3M Reference No. T-2541.1 (FC-3452), August, 1979

Ongoing Research/Study Protocols

1) Protocol, Pharmacokinetic Study of POSF in Rats, 3M Strategic Toxicology Laboratory, 3M Reference No. T-7098.1

Pre-1976 Studies (bibliography only)

1) Skin Irritation and Eye Irritation Study Report, WARF Institute, Inc., Project No. 5091241, 3M Reference No. T-1329, September 9, 1975

2) Acute Vapor Inhalation Toxicity Study in Rats, Industrial Bio-Test Laboratories, Project No. 663-07513, 3M Reference No. T-1329, October 23, 1975

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

2. FOSA	Perfluorooctanesulfonamide

Acute Toxicity

1) Acute Oral Toxicity Screen with T-3421 in Albino Rats, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883AR0287, 3M Reference No. T-3421 (KTZ-15), January 17, 1984

2) Acute Ocular Irritation Test with T-3421 in Albino Rabbits, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883EB286, 3M Reference No. T-3421 (KTZ-15), August 24, 1983

3) Primary Skin Irritation Test with T-3421 in Albino Rabbits, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883AR0288, 3M Reference No. T-3421 (KTZ-15), August 9, 1983

Studies in Progress

1) Protocol, Feces Method Development Metabolism Study for Perfluorooctanesulfonate Derivatives [N-EtFOSE, PFOS, and FOSA], 3M Strategic Toxicology Laboratory, Study Nos., T-636.17; T-6295.21; T-7132.3; ST-41, In-Life Start Date November 22, 1999, In-Life End Date November 24, 1999

2) Protocol, Pharmacokinetic Study of Perfluorooctane Sulfonamide [FOSA] in Rats, 3M Strategic Toxicology Laboratory, Study Nos., T132.2; ST-39, In-Life Start Date October 4, 1999, In-Life End Date November 2, 1999

3) Protocol, Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (PFOSA 3M T-7091.1) in Rats, Pathology Associates International, Study No. 1132-100

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

3. PFOSAA	Perfluorooctane sulfonylamido (ethyl)acetate

Acute Toxicity

1) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 77-1108A, 3M Reference No. T-1983 (FC-128, potassium salt 100%, solid), January 5, 1978

2) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

3) Primary Eye Irritation Study – Rabbits, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

4) Primary Skin Irritation Study – Rabbits, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

5) An Acute Inhalation Toxicity Study of T-2307 CoC in the Rat, Bio/dynamics, Inc., Project No. 78-7186, 3M Reference No. T-2307 (FC-128), February 8, 1979

Additional Acute Toxicity Studies Not Submitted (Bibliography only)

1) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129, approximately 40-50% in solution), March 2, 1978

2) Primary Eye Irritation Study – Rabbits, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129), March 2, 1978

3) Primary Skin Irritation Study – Rabbits, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129), March 2, 1978

4) Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088AR0362, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November 5, 1982

5) Primary Skin Irritation Test with T-3290CoC in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088EB0423, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), October 15, 1982

6) Acute Ocular Irritation Test with T-3290CoC in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088EB0424, 3M Reference No. T-

3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), October 26, 1982

Genotoxicity

1) Bacterial Reverse Mutation Test of ν-1, Hita Research laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute, Study Code: K01-1815, Report No. T-4663, 3M Reference No. T-6668.1, FC-129 (approximately 40-50% in solution of water and organic solvent), October, 1996

2) In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3290CoC, SRI International, Project No. 3145, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November, 1982

Pharmacokinetic Studies

1) 28 Dermal Percutaneous Absorption Study with FC-128 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0979AB0629, 3M Reference No. T-3991, March 15, 1981

2) 28 Day Dermal Percutaneous Absorption Study with FC-129 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0979AB0627, 3M Reference No. T-3989, March 14, 1981

3) Final Report – Analytical Study: Single-Dose Dermal Absorption / Toxicity Study of T-6051 and T-6054 in Rabbits, 3M Environmental Laboratory, Study No. ADMT-013195.1, in vivo Study Reference No. HWI 6329-133 (Hazleton Wisconsin, Inc.), 3M Reference Nos. T-6051 (FC-129 treated fabric) and T-6054 (FC-129 solution), November 22, 1995

4) Final Report, Analytical Report and Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits, 3M Environmental Technology & Services, In-Vivo Study Reference No. HWI#6329-138, Study No. AMDT-122094.2, 3M Reference No. FC-129, November 22, 1995

Studies in Progress

1) Corporate Toxicology Study Outline, FC-129 Preliminary ADME Screen in Rats, 3M Strategic Toxicology Laboratory, July, 1998

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

Pre-1976 Studies (bibliography only)

1) Skin and Eye Irritation Assay Report, WARF Institute, Project No. 2031046, 3M Reference No. FC-128, April 24, 1962 (plus December 29, 1966 letter containing individual eye scores)

4. PFDS	**Perfluorodecanesulfonate**

Acute Toxicity

1) Final Report, Acute Dermal Toxicity Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 21, 1988

2) Final Report, Acute Oral Toxicity Study in Rats, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 25, 1988

3) Final Report, Primary Eye Irritation/Corrosion Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 20, 1988

4) Final Report, Primary Dermal Irritation/Corrosion Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 21, 1988

Genotoxicity

1) Mutagenicity Test with T-6357 in the *Salmonella – Escherichia coli* / Mammalian-Microsome Reverse Mutation Assay, Corning Hazleton, Inc. (CHV), Project No. 17387-0-409, 3M Reference No. T-6357, FC-120, April 1, 1996

2) Mutagenicity Test on T-6357 in an In Vivo Mouse Micronucleus Assay, Corning Hazleton, Inc. (CHV), Project No. 17387-0-409, 3M Reference No. T-6357, FC-120, April 23, 1996

Pharmacokinetic Studies

1) Final Report, Analytical Report and Single-Dose Dermal Absorption / Toxicity Study of T-6052 in Rabbits, Hazleton Wisconsin, Inc., Project No. HWI 6329-135, 3M Reference No. FC-120, T-6052 (0.02 % in water), November 20, 1995

2) Single-Dose Dermal Intravenous Pharmacokinetic Study of T-6052 in Rabbits, Hazleton Wisconsin, Inc., Project No. HWI 6329-134, 3M Reference No. T-6052 (0.02 % in water), November 20, 19995

Attachments to Letter to C. Auer dated May 18, 2000
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Perfluorooctane Sulfonate-Related Compounds

Pre-1976 Studies (bibliography only)

1) Acute Oral Toxicity Study with T-1019 in Male Albino Rats, Industrial Bio-Test Laboratories, Inc., Project No. 601-05394, 3M Reference No. T-1019, August 6, 1974

2) Skin Irritation, Eye Irritation, Acute Oral LD50, WARF Institute, Inc., Project No. 4053863, 3M Reference No. T-992, May 24, 1974

3) Acute Oral Cholinesterase Study with T-1019 in Male Albino Rats, Industrial Bio-Test Laboratories, Inc., Project No. 601-05394, 3M Reference No. T-1019, August 6, 1974

5. PFHS	**Perfluorohexane sulfonate**

See confidential submission under letter of May 15, 2000 from Dr. John L. Butenhoff for one item including PFHS data

Attachments to Letter to C. Auer dated May 18, 2000
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Perfluorooctane Sulfonate-Related Compounds

6. N-EtFOSA	N-ethyl perfluorooctanesulfonamide

Acute Toxicity

1) Final Report, Acute Ocular Irritation Test with T-3608 in Albino Rats, Riker Laboratories, Inc., 3M Reference FX-12, Study No. 0984EB0367, September 5, 1984

2) Final Report, Primary Skin Irritation Test with T-3608 in Albino Rats, Riker Laboratories, Inc., 3M Reference FX-12, Study No. 0984EB0368, August 13, 1984

3) Final Report, Acute Oral Toxicity Screen with T-3066CoC in Albino Rats, Riker Laboratories, Inc., 3M Ref. No. FX-12, Study No. 0981AR0146, July 13, 1981

Acute Toxicity Studies Not Submitted (Bibliography Only)

1) Final Report, Acute Oral Toxicity Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101149, January 31, 1997

2) Final Report, Primary Dermal Irritation/Corrosion Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101150, January 31, 1997

3) Final Report, Primary Eye Irritation/Corrosion Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101151, January 31, 1997

Genotoxicity

1) Final Report, Protocol and two amendments, Mutagenicity Test on T-6294 in an In Vivo Mouse Micronucleus Assay, Corning Hazelton, Inc., Study No. 1785-0-455, May 10, 1996

Previously submitted with May 4, 2000 letter, Advanced Bioanalytical Services, Inc., Analytical Report, Additional Characterization of Metabolites of T-6292, T-6293 and T-6294 from Rat and Human Hepatocytes by TurboIonSpray LC/MS and LC/MS/MS. Semi-Quantitative Analysis of T-6295 in Rat and Human Hepatocytes Incubated with T-6292, T-6293 and T-6294 by LC/MS/MS, January 28, 1998, Report 98AGKP01.3M

Attachments to Letter to C. Auer dated May 18, 2000
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Perfluorooctane Sulfonate-Related Compounds

Mechanistic

1) T. J. Cross and R. G. Schnellmann, Mechanism of Toxicity of a Unique Pesticide N-Ethylperfluorooctane Sulfanomide (NEPFOS), and its metabolite perfluorooctane Sulfonamide (PFOS) to Isolated Rabbit Renal Cortical Mitochondria (RCM), Abstract from 1989 Society of Toxicology Meeting

Previously submitted with May 4, 2000 letter - Qualitative Investigation of the In Vitro Metabolism of T-6292 (n-ethyl FOSE), T-6293 (n-ethyl FOSE phosphate diammonium salt(ester)), T-6294 (n-ethyl perfluorooctane sulfonamide) and T-6295 (perflurooctane sulfonate) by Rat and Human Hepatocytes Using Ion Spray LC/MS and LC/MS/MS, Advanced Bioanalytical Services, Inc., [Preliminary] Analytical Report, Report 96ADEM01.3M, November 12, 1996

Previously submitted with May 4, 2000 letter - Advanced Bioanalytical Services, Inc., Analytical Report, Additional Characterization of Metabolites of T-6292, T-6293 and T-6294 from Rat and Human Hepatocytes by TurboIonSpray LC/MS and LC/MS/MS. Semi-Quantitative Analysis of T-6295 in Rat and Human Hepatocytes Incubated with T-6292, T-6293 and T-6294 by LC/MS/MS, January 28, 1998, Report 98AGKP01.3M

Analytical

1) Analytical and Research Properties – 3M Industrial Hygiene Laboratory, January 1993

6. N-EtFOSA	N-ethyl perfluorooctanesulfonamide

Bibliography Showing Studies in 3M's Possession Believed To Be In FIFRA Docket.

REDACTED

7. N-MeFOSA	N-methyl perfluorooctanesulfonamide

Acute Toxicity

1) Acute Oral Toxicity – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503499, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), July 12, 1985, with Protocol

2) Primary Dermal Irritation – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503500, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June 24, 1985, with Protocol

3) Primary Eye Irritation – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503501, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June 24, 1985, with Protocol

4) Acute Oral Toxicity – Method, Summary, Pathology; Primary Dermal Irritation – Method, Summary; Primary Eye Irritation – Method, Summary; QAU Report; Raw Data Appendix, Hazleton Laboratories America, Inc,. Project No. 50202473, 3M Reference No. T-3727 (F-10034, Lot 7, distilled wide-range), May 7, 1985, with Protocol

5) Acute Oral Toxicity Screen with T-3065CoC in Albino Rats, Riker Laboratories, Inc., Experiment No. 0981AR0145, May 15, 1981

Genotoxicity

1) In Vitro Microbiological Mutagenicity Assays of T-3752, SRI International, Project No. LSC-3145, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June, 1985

2) In Vitro Microbiological Mutagenicity Assays of T-3727, SRI International, Project No. LSC-3145, 3M Reference No. T-3727 (F-10034, Lot 7, distilled wide-range), March, 1985

8. N-EtFOSE alcohol	**N-ethyl perfluorooctane sulfonamidoethanol**

Acute Toxicity

1) Corrected Final Report, Acute Inhalation Toxicity Study in Rats, 3M Reference No. T-2991IT, Hazelton Laboratories America, Inc., Study No. 154-157, February 2, 1981

Genotoxicity

1) Final Report, Mutagenicity Test on T-5710 in an *In Vivo* Rat Micronucleus Assay, Hazelton Washington, Inc., Study No. 15516-0-454, April 23, 1993

2) Final Report, Genotoxicity Test on T-5710.1 in the In Vivo/In Vitro Unscheduled DNA Synthesis and Cell Proliferation in Rat Liver Cells, Hazelton Washington, Inc., Study No. 15516-0-494, September 14, 1993

3) Final Report, Mutagenicity Test on T-6292 in an *In Vivo* Mouse Micronucleus Assay, Hazelton Washington, Inc., Study No. 17384-0-455, May 2, 1996, with Protocol and Protocol amendments

4) Evaluation of the Mutagenic Activity of T-6906 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells, NOTOX Study No. 223458, 3M Reference No. FM-3923

   a) Final Report, December 22, 1998 (see letter below)

   b) Letter from Steve R. Haworth, Ph.D., Covance Laboratories, reviewing the NOTOX study and concluding it was technically inadequate and should be repeated
   Note: 3M is repeating the study

Repeated-Dose Toxicity

1) Final Report, Ninety Day Subacute Rat Toxicity Study on FM-3422, International Research and Development Corporation, Study No. 137-086, November 10, 1978

2) Final Report, Ninety Day Subacute Rhesus Monkey Toxicity Study, on FM-3422, International Research and Development Corporation, Study No. 137-088, January 16, 1979

3) Two-Year Dietary Study

   a) Report, Volumes 1-5, Two Year Oral (Diet) Toxicity / Carcinogenicity Study of Fluorochemical FM-3924 in Rats, Riker Laboratories, Inc., Study No. 0281CR0012, August 29, 1987, Conducted during April 1981 - May 1983

   b) Report Amendment No. 1, Two Year Oral (Diet) Toxicity / Carcinogenicity Study of Fluorochemical FM-3924 in Rats, Riker Laboratories, Inc., Study No. 0281CR0012, October 25, 1988

   c) Xenos Letter dated May 24, 1991 with attachment: 3M Response to FDA Letter of December 10, 1990 [re 2 year cancer study], Food Additive Petition Nos. 0B4197 and 0B4206

   d) Pathology Review of Reported Tumorigenesis in a Two Year Study of FM-3924 in Rats, Pathology Associates International, November 25, 1998

   e) Analytical on retained sample

   f) Review by Dr. William H. Butler of Two Year (Diet) Toxicity / Carcinogenicity Study of Fluorochemical FM 3924 in Rats

4) Ongoing 2-Year Study

   a) Summary Report of Week 53, 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfanoamido Ethanol in Rats, 3M Reference No. T-6316.1, Covance Laboratories, 6329-212 and –228 (draft final report expected fall 2000)

<u>Pharmacokinetic Studies</u>

1) Synthesis and Characterization of N-Ethyl FOSE ^{14}C, Riker Laboratories, March 17, 1981

2) Final Report, Absorption and Biotransformation of N-Ethyl FOSE and Tissue Distribution and Elimination of Carbon-14 after Administration of N-Ethyl FOSE - ^{14}C in Feed, Riker Laboratories, Inc., January 19, 1983

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

Previously submitted with May 4, 2000 letter -- Qualitative Investigation of the In Vitro Metabolism of T-6292 (n-ethyl FOSE), T-6293 (n-ethyl FOSE phosphate diammonium salt(ester)), T-6294 (n-ethyl perfluorooctane sulfonamide) and T-6295 (perflurooctane sulfonate) by Rat and Human Hepatocytes Using Ion Spray LC/MS and LC/MS/MS, Advanced Bioanalytical Services, Inc., [Preliminary] Analytical Report, Report 96ADEM01.3M, November 12, 1996

Previously submitted with May 4, 2000 letter - Advanced Bioanalytical Services, Inc., Analytical Report, Additional Characterization of Metabolites of T-6292, T-6293 and T-6294 from Rat and Human Hepatocytes by TurboIonSpray LC/MS and LC/MS/MS. Semi-Quantitative Analysis of T-6295 in Rat and Human Hepatocytes Incubated with T-6292, T-6293 and T-6294 by LC/MS/MS, January 28, 1998, Report 98AGKP01.3M

Mechanistic

1) Final Report, Analysis of T-5877 in a Cell Proliferation Assay in Rat Liver Cells, Hazelton Washington, Inc., Study No. 154-208, 3M Reference T-5877 (wide range ethyl FOSE), FM-3924, Lot 547, L-13202, November 1, 1994, with Protocol and Protocol Addendum, Key to FC Alcohol Tox Samples (including 5877), and Analytical data

Teratology

1) Oral (Gavage) Developmental Toxicity Study of N-EtFOSE in Rats

   a) Final Report, Oral (Gavage) Developmental Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.7, December 17, 1998

   b) Summary N-Etfose Rat Teratology, Oral (Gavage) Developmental Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.7

2) Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits

   a) Final Report, Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits, 3M Reference No. T-6316.8, January 11, 1999

   b) Summary N-EtFOSE Rabbit Teratology, Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits, 3M Reference No. T-6316.8

3) Teratology Studies of T-2999 (FM-3924) in Rabbits

a) Final Report, Oral Rangefinder Study of T-2999CoC in Pregnant Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Study No. 0680RB0019, June 25, 1981

b) Final Report and Protocol, Oral Teratology Study of T-2999CoC in Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Study No. 0681TB0212, 3M Reference No. FM 3924 (88% ethyl FOSE), January 7, 1982

c) 3M Internal Correspondence re alcohols being used in Riker studies, from DR Ricker to WC McCormick, dated December 10, 1980

d) Analytical Analyses of Suspension of FM-3924, Internal Memo, from TR Mathisen to EG Gortner, dated April 8, 1981

e) 3M Internal Correspondence, Analytical Evaluation of FM-3924, dated June 22, 1982

4) Oral Teratology Study of FM-3422 in Rats

a) Final Report, Oral Teratology Study of FM-3422 in Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Study No. 0680TR0010, January 22, 1981

b) 3M Internal Correspondence re alcohols being used in Riker studies, from DR Ricker to WC McCormick, dated December 10, 1980

5) Teratology Studies of T-2999CoC (FM-3924) in Rats

a) Final Report, Special Lens Oral Teratology Study of T-2999CoC in Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Study No. 0680TR0020, 3M Reference No. FM-3924, December 22, 1981

b) Final Report and Protocol, Special Lens Oral Teratology Study of T-2999CoC in Two Strains of Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Study No. 0681TR0362, July 20, 1982

c) 3M Internal Correspondence re alcohols being used in Riker studies, from DR Ricker to WC McCormick, dated December 10, 1980

d) Analytical Analyses of Suspension of FM-3924, Internal Memo, from TR Mathisen to EG Gortner, dated April 8, 1981

e) 3M internal correspondence re analytical analysis of FM-3924, from EG Gortner to RM Payfer, dated June 22, 1982

6) Memo from EG Lambrecht to Riker Study Files re Fetal Lens Artifact -- Summary of Developments to Date, dated November 6, 1981

Previously submitted to TSCA 8e docket, Final Report, Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.5, June 30, 1999
Note: weight gain effects in F_1 generation currently being reanalyzed. Report may require amendment.

Studies In Progress

1) Protocol, N-EtFOSE Bile Method Development in Rats, 3M Strategic Toxicology Laboratory, Study No. T-6316.15; ST-30, In-Life Start Date August 12, 1999, In-Life End Date August 20, 1999

2) Protocol, Feces Method Development Metabolism Study for Perfluorooctanesulfonate Derivatives [N-EtFOSE, PFOS, and FOSA], 3M Strategic Toxicology Laboratory, Study Nos., T-636.17; T-6295.21; T-7132.3; ST-41, In-Life Start Date November 22, 1999, In-Life End Date November 24, 1999

3) Protocol, Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (PFOSA 3M T-7091.1) in Rats, Pathology Associates International, Study No. 1132-100

Pre-1976 Studies (bibliography only)

1) Final Report, Oral LD 50 (4 levels), 3M Reference No. T-961, WARF Institute, Inc., Study No. 4043911, June 12, 1974

2) Final Report, Acute Vapor Inhalation Toxicity Study in Rats, 3M Reference No. T-1260, Industrial Bio-Test Laboratories, Inc., July 21, 1975

3) Final Report, Acute Vapor Inhalation Toxicity Study in Rats, 3M Reference No. T-1259, Industrial Bio-Test Laboratories, Inc., July 21, 1975

4) Final Report, Skin and Eye Irritation Study, WARF Institute, Inc., Study No. 5060080, 3M Reference No. T-1260, June 17, 1975

5) Final Report, Skin and Eye Irritation Study, WARF Institute, Inc., Study No. 5060079, 3M Reference No. T-1259, June 17, 1975

9. N-MeFOSE alcohol	**N-methylperfluorooctane sulfonamidoethanol**

Acute Toxicity

1) Acute Oral Toxicity – Rats, Biosearch, Inc., 3M Ref. T-2038CoC (L-4309, FC-5160) (75% in 25% $CaCO_3$), Study No. 78-1161A, Feb. 2, 1978

2) Primary Eye Irritation Study – Rabbits, Biosearch, Inc., 3M Ref. T-2038CoC (L-4309, FC-5160) (75% in 25% $CaCO_3$) , Study No. 78-1161A, Feb. 2, 1978

3) Primary Skin Irritation Study – Rabbits, Biosearch, Inc., 3M Ref. T-2038CoC (L-4309, FC-5160) (75% in 25% $CaCO_3$), Study No. 78-1161A, Feb. 2, 1978

4) Acute Oral Toxicity Screen with T-2574CoC in Albino Rats, Riker Laboratories, Inc., Experiment 0979AR0037, September 26, 1979

Genotoxicity

1) Genotoxicity Test on T-5711.1 in the In Vivo/In Vitro Unscheduled DNA Synthesis and Cell Proliferation Assays in Rat Liver Cells, Hazelton Washington, HWA Study No. 15515-0-494, September 14, 1993, and attached protocol

2) Mutagenicity Test on T-5711 in an In Vivo Rat Micronucleus Assay, Hazelton Washington, HWA Study No. 15515-0-454, April 30, 1993, and attached Protocol and memorandum containing analytical data

3) Evaluation of the Mutagenic Activity of T-5874 in the Ames Salmonella/Microsome Test (with Independent Repeat), NoTox Project 115932, NoTox Substance 38187, 3M Reference T-5874.1, April 19, 1994

4) Evaluation of the Ability of T-5874 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (with Independent Repeat), NoTox Project 115919, NoTox Substance 38187, 3M Reference T-5874.2, May 5, 1994

5) Evaluation of the Mutagenic Activity of T-5874 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells (with Independent Repeat), NoTox Project 115921, NoTox Substance 38187, 3M Reference T-5874.3, April 19, 1994

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Repeated-Dose Toxicity

1) 13-Week Dietary Toxicity Study with N-Methyl Perfluorooctanesulfonamide Ethanol (N-MeFOSE, T-6314) in Rats, Covance Study No. 6329-225, 3M Reference No. T6314.1

   a) Audited Draft Report, May 25, 1999
   b) Letter from Andrew M. Seacat, Ph.D., Study Monitor, to Peter J. Thomford, Ph.D., Study Director at Covance, regarding errors in draft report

Mechanistic

1) Analysis of T-5794 in a Cell Proliferation Assay in Rat Liver Cells, Hazelton Washington, Inc., Final Report, HWA Study No. 154-207, January 26, 1994, and protocol and analytical data

2) Analysis of T-5878 in a Cell Proliferation Assay in Rat Liver Cells, Hazelton Washington, Inc., Final Report, HWA Study No. 154-209, November 1, 1994, and protocol and analytical data

10. N-EtFOSEA	**N-ethylperfluorooctane sulfonamidoethyl acrylate**

Acute Toxicity

1) Acute Oral Toxicity Screen with T-3493 in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0884AR0010, 3M Reference No. T-3943, February 7, 1984

2) Primary Skin Irritation Test with T-3493 in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0884EB0009, 3M Reference No. T-3943, February 7, 1984

3) Acute Ocular Irritation Test with T-3493 in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0884EB0008, 3M Reference No. T-3943, January 24, 1984

Immunotoxicity

1) Guinea Pig Maximization – Method, Summary, and Raw Data Appendix, Hazleton Laboratories America, Inc., Project No. 40703984, 3M Reference No. T-3609, October 5, 1984 (plus May 13, 1985 report amendment)

Genotoxicity

1) Chromosomal Aberration Study of Sample D-1 in Cultured Mammalian Cells, Mitsubishi Chemical Safety Institute, Ltd., Study No. 2L162, 3M Reference No. T-6322.3 (FX-13), October 23, 1995

2) Mutagenicity Testing of 2-[N-ethyl-N-perfluoroalkyl (C=1~8)sulfonylamino]ethyl acrylate in Bacterial Reverse Mutation Assays, BML, Inc., Study No. 2862, 3M Reference No. T-6322.5 (FX-13), April 24, 1996

3) In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3609, SRI International, Project No. LSC-3145, September, 1984

Repeated-Dose Toxicity

1) Twenty-Eight-Day Repeated Dose Oral Toxicity Study of Sample D-1 in Rats, Bio-Medical Research Laboratories Co., Ltd., Study No. BMR143C, 3M Reference No. T-6322 (FX-13), February 16, 1993

11. N-EtFOSEMA	**N-ethyl perfluorooctane sulfonamido ethyl methacrylate**

Acute Toxicity

1) Acute Oral Toxicity Screen with T-3494 in Albino Rats, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0884AR0013, 3M Reference No. T-3494 (L-1048, FX-14), March 13, 1984

2) Primary Skin Irritation Test with T-3494 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0884EB0012, 3M Reference No. T-3494 (L-1048, FX-14), February 7, 1984

3) Acute Ocular Irritation Test with T-3494 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0884EB0011, 3M Reference No. T-3494 (L-1048, FX-14), January 24, 1984

Immunotoxicity

1) Guinea Pig Maximization, Hazleton Laboratories America, Inc., Project No. 40703985, 3M Reference No. T-3610 (NB No. 63601-32, FX-14), October 9, 1984

Genotoxicity

1) In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3610, SRI International, Project No. LSC-3145, 3M Reference No. T-3610 (NB No. 63601-32, FX-14), September, 1984

12. N-MeFOSEA	**N-methylperfluorooctane sulfonamidoethyl acrylate**

Genotoxicity

1) Evaluation of the Mutagenic Activity of T-5869 in the Ames Salmonella/Microsome Test (with independent repeat), NOTOX, Project No. 115965, 3M Reference No. T-5869.1 (FMZ-3559, Lot 2408, wide-range), April 20, 1994

2) Evaluation of the Ability of T-5869 to Induce Chromosome Aberrations in Cultured Human Lymphocytes (with independent repeat), NOTOX, Project No. 115943, 3M Reference No. T-5869.2 (FMZ-3559, Lot 2408, wide-range), June 5, 1994

3) Evaluation of the Mutagenic Activity of T-5869 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells (with independent repeat), NOTOX, Project No. 115954, 3M Reference No. T-5869.3 (FMZ-3559, Lot 2408, wide-range), April 20, 1994

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Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

13. PFOS	Additional Studies on Perfluorooctane Sulfonate

Acute Toxicity

1) Acute Toxicity Tests for T-T-6684, Didecyldimethylammonium salt of perfluorooctanesulfonate:

   a) Acute Oral Toxicity Study of T-6684 in Rats (OECD Guidelines), Corning Hazleton, Inc., Project No. CHW 61101149, 3M Reference No. T-6684 January 31, 1997

   b) Primary Dermal Irritation / Corrosion Study of T-6684 in Rabbits (OECD Guidelines), Corning Hazleton, Inc., Project No. CHW 61101150, 3M Reference No. T-6684 (didecyldimethylammonium salt of perfluorooctanesulfonate), January 10, 1997

   c) Primary Eye Irritation / Corrosion Study of T-6684 in Rabbits (OECD Guidelines), Corning Hazleton, Inc., Project No. CHW 61101151, 3M Reference No. T-6684 (didecyldimethylammonium salt of perfluorooctanesulfonate), January 28, 1997

2) Acute Toxicity Tests for T-5898, lithium perfluorooctane sulfonate, 3M Ref. FC-94:

   a) Final Report, Acute Oral Toxicity Study of T-5898 in Rats, Hazelton Wisconsin, Study No. 40200468, April 22, 1994

   b) Final Report, Primary Eye Irritation/Corrosion Study of T-5898 in Rabbits, Hazelton Wisconsin, Study No. 40200469, April 7, 1994

   c) Final Report, Primary Dermal Irritation/Corrosion Study of T-5898 in Rabbits, Hazelton Wisconsin, Study No. 40200470, March 23, 1994

3) Acute Oral Toxicity – Rats, Biosearch, Inc., 3M Reference No. T-1388 (perfluorooctanesulfonic acid), March 4, 1976

Pharmacokinetic Studies

1) Draft Report, 5-Daily Dose Dermal Absorption / Toxicity Study of T-6684 in Rabbits, Covance Laboratories, Inc., Study No. 6329-200, 3M Reference No. T-6684 (didecyldimethylammonium salt of perfluorooctanesulfonate, slurry), July 11, 1997

2) Qualitative Investigation of the In Vitro Metabolism of T-6292 (n-ethyl FOSE), T-6293 (n-ethyl FOSE phosphate diammonium salt(ester)), T-6294 (n-ethyl perfluorooctane sulfonamide) and T-6295 (perflurooctane sulfonate) by Rat and Human Hepatocytes Using Ion Spray LC/MS and LC/MS/MS, Advanced Bioanalytical Services, Inc., [Preliminary] Analytical Report, Report 96ADEM01.3M, November 12, 1996

Teratology

1) Memorandum from E. G. Lamprecht re Fetal Rat Lens Artifact – Summary of Developments to Date, Nov. 6, 1981

Analytical

Ion Spray LC/MC Determination of Perfluoro Analytical Standards Provided by 3M Medical Department, Advanced Bioanalytical Services, Inc., No. 95MYHW01.3M, August 30, 1995

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2) Protocol, Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (PFOSA 3M T-7091.1) in Rats, Pathology Associates International, Study No. 1132-100

13. PFOS	Additional Studies on Perfluorooctane Sulfonate

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3. PFOSAA – Perfluorooctane sulfonylamido (ethyl) acetate

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4. PFDS - Perfluorodecanesulfonate

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5. PFHS - Perfluorohexanesulfonate

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Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

1. POSF	Perfluorooctanesulfonyl fluoride

Acute Toxicity

1) Primary Skin Irritation Test with T-3874 in Albino Rabbits, Pathology and Toxicology, Riker Laboratories, Project No. 0386EB0135, 3M Reference No. T-3874, April 14, 1986

2) T-3607 Acute Inhalation Toxicity Test, Bushy Run Research Center, Project No. 47-527, 3M Reference No. T-3607, December 19, 1984

3) Acute Oral Toxicity Study – Method, Summary, Pathology and Raw Data Appendix, Hazleton Laboratories America, Inc., Project No. 40703983, October 5, 1984

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Primary Skin Irritation Test
with T-3874
in Albino Rabbits

Experiment No: 0386EB0135

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: March 18, 1986 to March 20, 1986

Conducted By: Gene L. Harris 4/14/86
G. L. Harris, BS Date
Advanced Toxicologist
Study Director

Reviewed By: C. F. Chesney 4/14/86
C. F. Chesney, D.V.M., Ph.D. Date
Manager, Pathology and Toxicology

dc: K. L. Ebbens
F. D. Griffith
R. G. Perkins

Summary

The results of the primary skin irritation test conducted from March 18, 1986 to March 20, 1986 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3874 is non-irritating (0.0/8.0) to the skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

The objective of this study was to determine the primary skin irritation potential of T-3874 to the skin of female albino rabbits. This test was conducted to meet the Department of Transportations requirements for primary skin irritation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Animals and Husbandry

Young New Zealand White Rabbits[a] were used in the evaluation of the primary skin irritating properties of the test article. The rabbits were individually housed[b] in stainless steel cages, and food[c] and water were available ad libitum. All rabbits were individually identified with ear tags and considered to be in good health at study initiation. The rabbits were housed in a temperature and humidity controlled room. Room lighting was on a 12/12 hour light/dark cycle that was automatically timed.

Method and Results

The test procedure was modeled after that of Draize et al[d]. One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and the test site was selected lateral to the midline of the back approximately ten centimeters apart.

The test article (0.5 ml) was applied to the intact test site on each rabbit and immediately covered with two-inch square gauze patches. The patch, which was placed directly over the test site, was secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[e] which held the patches in position during the four hour exposure period.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Animals were housed in accordance with recommendations contained in DHEW Publication No. 78-23 (NIH): Revised 1978 "Guide For the Care and Use of Laboratory Animals.
[c] Purina Lab Rabbit Chow® and rabbits may be offered Alfalfa Cubes for additional roughage.
[d] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[e] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, IL

At the end of four hours, the plastic wrappings, patches, and all residual test article were removed by washing with water. One hour and two days after removal of the test article, the abraded test site was examined and scored for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the abraded test site one hour and two days post removal of the test article. This value was divided by two to obtain the mean primary irritation index. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-Irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with T-3874

Animal Number	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	Day 2 Er.	Day 2 Ed.
6B0394	0	0	0	0
6B0397	0	0	0	0
6B0389	0	0	0	0
6B0392	0	0	0	0
6B0395	0	0	0	0
6B0398	0	0	0	0
Mean	0.0	0.0	0.0	0.0

Subtotal 0.0

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No.: 0386EB0135

PROTOCOL

TEST: Acute Primary Skin Irritation Test

SPONSOR: **3M** Commercial Chemical **Division**

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-3874

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 3/86 - 6/86

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: HAZLETON - DUTCHLAND
DENVER, PA

OBJECTIVE: To determine the irritation potential of the test article to the skin of six animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and one test sites selected lateral to the midline of the back approximately ten centimeters apart. None of the one sites will be abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 ml) will be applied to no abraded and one intact site(s) on each animal, covered with gauze and secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the[c] day exposure period. One hour and 48 hours after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4[b]. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States

[c] 4-hour exposure period; for DOT see attached from 49CFR173.

Sponsor — 8/27/86 Date

Study Director — 2/28/86 Date

Form 19171-12-B-PWO

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. L. Harris, BS	Advanced Toxicologist Study Director
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect differenct significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

UNION CARBIDE

BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 733-5200

PROJECT REPORT 47-527

TITLE: T-3607
Acute Inhalation Toxicity Test

AUTHOR: Donald J. Nachreiner

SPONSOR: 3M Company
3M Center
220-2E-02
St. Paul, MN 55144

INITIATOR: William C. McCormick

DATE: December 19, 1984

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R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 733-5200

Project Report 47-527
11 Pages
December 19, 1984

T-3607
Acute Inhalation Toxicity Study

Sponsor: 3M Company

* * * * *

Abstract

Five male and five female Wistar albino rats were exposed for four hours to a vapor of T-3607. The actual chamber concentration was 3.74 mg/L (182 ppm). There were no mortalities or clinical signs of toxicity during the exposure or postexposure periods. Mean body weight gain was observed for both sexes during the postexposure period. There were no gross pathologic lesions. The results of this study indicate that the four-hour LC50 for T-3607 is greater than 3.74 mg/L.

Objective

This study was designed to determine the acute inhalation toxicity to rats resulting from a single, four-hour exposure to T-3607.

Materials and Methods

This study followed the specific protocol (BRRC Project 84-62-40122) and standard amendment to the protocol prepared by the Bushy Run Research Center.

Test Article

A two-quart bottle of T-3607 was received on August 9, 1984 from 3M Company (St. Paul, MN), assigned BRRC Sample Number 47-249, and stored in Rooms 109 and 118. Information received from the Sponsor stated the purity to be approximately 100% perfluorooctyl sulfonyl fluoride. An identificaton or CAS Registry Number was not available from the Sponsor.

Animal Species, Source and Husbandry

Male and female Hilltop-Wistar albino rats [200-300 g, HLA(WI)BR] (Hilltop Lab Animals, Inc., Scottdale, PA) were used. On the day of exposure the male and female rats were 45 and 59 days of age, respectively. They were received on September 19, 1984, and assigned unique identification numbers by toe-clipping. The rats were housed five per sex in 23.5 x 40.0 x 18.0 cm high stainless steel wire mesh cages on carriers in Room 109 and kept on a 12-hour photoperiod throughout the postexposure period. A layer of Deotized Animal Cage Board® (Shepherd Specialty Papers, Inc., Kalamazoo, MI) was placed under each row of cages. Pelleted feed (Agway Prolab RMH3000 Certified Rodent Chow, Agway Inc., Syracuse, NY) and tap water (Municipal Authority of Westmoreland County, Greensburg, PA) were available ad libitum except during exposure.

Inhalation Chamber Design and Operational Characteristics

The rats were individually housed in 16.5 x 9.5 x 15.0 cm wire mesh cages and exposed in a 120-liter (approximate volume) cuboidal Plexiglas® chamber in Room 118. Total airflow through the chamber was maintained at approximately 25 liters per minute. The inhalation chamber design and operation are summarized in Table 1.

Exposure Regimen

Five male and five female rats were exposed once for four hours on September 24, 1984, to a vapor atmosphere of T-3607. No control exposures were performed.

Generation of the Test Material

The T-3607 was metered with a piston pump (FMI Model RPG-6/Lab Pump Jr. Assembly) into a glass tube containing glass beads used to facilitate evaporation at ambient temperature. The fluid was metered at a rate that would provide a nominal concentration of approximately 5 mg/L. Dry, filtered, compressed air was used to vaporize the test material. The entire chamber airflow (approximately 25 liters per minute) was passed through the evaporation tube. Figure 1 presents a schematic diagram of the generation and exposure system.

Air was removed from the chamber via a vacuum line. The exhaust air was filtered through three scrubbing devices each containing approximately two liters of water.

Target Concentration

The target concentration for this exposure was 5 mg/L, based on the guidelines for "limit" testing set forth by the Toxic Substances Control Act (TSCA).

Chamber Concentration Analysis

The concentration of T-3607 in the chamber was measured approximately every 30 minutes. A known volume of air was sampled from the breathing zone of the animals through evacuated flasks supplied by the 3M Company. The flasks containing the atmosphere samples of T-3607 were sent to the Sponsor for analysis of perflurooctyl sulfonyl fluoride.

The nominal concentration was determined by dividing the total weight of the test material used by the total volume of air which passed through the chamber during the exposure period.

Temperature and Relative Humidity

The temperature and relative humidity in the animal housing room were recorded continuously with a seven-day recording hygrothermograph (Cole Parmer Instruments, Chicago, IL). During the exposure the temperature was monitored with a Series 400 A Digital Trendicator (Doric Scientific, San Diego, CA) and the humidity was monitored with a Model 8501 A Humidity Module (Hygrometrix Inc., Oakland, CA).

Animal Observation

All animals were observed during exposure, and for 14 days following exposure for signs of toxic effects.

Body Weights

The animals were weighed prior to exposure and on postexposure days seven and fourteen. The change in body weight was calculated by subtracting the pre-exposure values from each successive weight.

Necropsy

The animals were sacrificed on October 8, 1984. Following methoxyflurane anesthesia, the animals were exsanguinated by severing the brachial blood vessels. A complete necropsy was performed. No tissues were saved.

Statistical Procedures

The mean and standard deviations of the body weights, body weight changes, and exposure concentrations, were calculated. No statistical comparisons were made.

Storage of Records

The final report and all raw data are retained in the Archives of the Bushy Run Research Center.

Results

Chamber Concentration

The mean (± standard deviation) measured concentration of perfluorooctyl sulfonyl fluoride (T-3607 is approximately 100% POSF) in the exposure chamber was 3.74 (± 0.26) mg/L. The individual data for the actual exposure concentrations received from the Sponsor are presented in Table 2. The nominal concentration was 5.2 mg/L.

Humidity and Exposure Chamber Conditions

The temperature and relative humidity in the animal housing room ranged between 21 and 22°C and 43 and 57% respectively, throughout the study period. The mean (± standard deviation) temperature and humidity in the exposure chamber was 26 (± 0)°C and 14 (± 5)%, respectively.

Animal Observations

There were no clinical signs of toxicity observed during the exposure or during the 14-day postexposure period.

Mortality

No rats died during exposure or during the 14-day postexposure period.

Body Weights

The individual body weights and body weight changes are summarized in Table 3. Mean body weight gains were observed for both sexes on postexposure days 7 and 14.

Necropsy

No gross pathologic lesions were found in any animals.

Discussion

A single four-hour exposure to an actual chamber concentration of 3.74 mg/L T-3607 produced no mortality. There were no clinical signs observed during exposure or during the 14-day postexposure period. Both sexes gained weight during the postexposure period. There were no gross pathologic lesions. The results of this study indicate that the LC50 for T-3607 vapor in Wistar albino rats is greater than 3.74 mg/L.

Project Team

D. J. Nachreiner, B.S.	Study Director
M. L. Steel	Senior Technologist
D. R. Klonne, Ph.D.	Project Manager

Reviewed and Approved by:

Donald J Nachreiner 12-11-84

Donald J. Nachreiner, B.S. Date
Study Director

Dennis R Klonne 12-11-84

Dennis R. Klonne, Ph.D. Date
Project Manager

F. R. Frank 12/17/84

Fred R. Frank, Ph.D. Date
Director

WPC/rkk/0630B-2
11-19-84

Table 1

Inhalation Chamber Design and Operation

T-3607 Acute Inhalation Toxicity Test

CHAMBER

Location: Room 118

Construction: The chamber was made of Plexiglas®.

Shape: Cuboidal

Dimensions: Height - 0.38 meter
Length - 0.61 meter
Width - 0.52 meter
Total Volume - Approximately 120 liters

Airflow Measurement: A Manostat flowmeter, calibrated with a Singer® dry test meter, was positioned in the air supply line to the evaporator tube.

Test Article Generation: FMI Model RPG-6/Lab Pump Jr. Assembly; glass evaporator tube containing glass beads; ambient temperature.

Chamber Atmosphere Conditions:

Chamber Number	Nominal Concentration (mg/L)	Temperature* (°C) Mean ± SD	Relative Humidity** (%) Mean ± SD	Air Flowrate (L/min)
120-1	5.2	26 ± 0	14 ± 5	25

* Determined using a Series 400 A Digital Trendicator.
**Determined using a Model 8501 A Humidity Module.

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11-19-84

Table 2

Exposure Concentration Measurements

T-3607 Acute Inhalation Toxicity Test

Actual Concentrations:

Sample Number	Time into Exposure (min)	Sample Flowrate L/min)	Sample Duration (min)	POSF* Concentration (mg/L)	POSF* Concentration (ppm)
A	55	1	5	3.45	168
B	65	1	5	3.47	169
C	115	1	5	3.65	178
D	125	1	5	3.48	169
E	175	1	5	3.94	192
F	185	1	5	3.87	188
G	210	1	5	3.99	194
H	215	1	5	4.11	200
			Mean	3.74	182
			Standard Deviation	0.26	13

Nominal Concentration:

Target Concentration (mg/L)	Exposure Duration (min)	Chamber Airflow (L/min)	Total Mass of T-3607 Used (grams)	Nominal Concentration (mg/L)
5.0	240	25	31**	5.2

* The T-3607 samples were analyzed by the sponsor for the concentration of perfluorooctyl sulfonyl fluoride (T-3607 is approximately 100% POSF).

** A volume of 17.2 ml T-3607 was metered. At ambient temperature, 31 grams of T-3607 (17.2 ml*density of 1.8 gm/ml) was vaporized. The density was determined by weighing 10 ml of T-3607.

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Table 3
Individual Animal Data
T-3607 Acute Inhalation Toxicity Test

Animal Number	Weight At Day 0	7	14	Body Wt. Change 7	14	Gross Pathology at Necropsy
			MALES*			
84-14763	241	270	306	29	65	NGL
84-14764	246	284	324	38	78	NGL
84-14765	253	304	348	51	95	NGL
84-14766	245	295	353	50	108	NGL
84-14767	258	329	378	71	120	NGL
Mean:	249	296	342	48	93	Mortality
Std. Dev.:	7	22	28	16	22	Ratio 0/5
Group Observations:	No clinical signs were observed during the 4-hr vapor exposure or 14-day postexposure periods.					
			FEMALES*			
84-14828	241	266	276	25	35	NGL
84-14829	241	243	257	2	16	NGL
84-14830	251	263	282	12	31	NGL
84-14831	243	256	267	13	24	NGL
84-14832	228	249	252	21	24	NGL
Mean:	241	255	267	15	26	Mortality
Std. Dev.:	8	10	13	9	7	Ratio 0/5
Group Observations:	No clinical signs were observed during the 4-hr vapor exposure or 14-day postexposure periods.					

NGL = No Gross Lesions
Exposure Date: September 24, 1984
*Albino rats [HLA(WI)BR] Hilltop Lab Animals, Inc., Scottdale, PA were used.

WPC/rkk/0630B-3
12-10-84

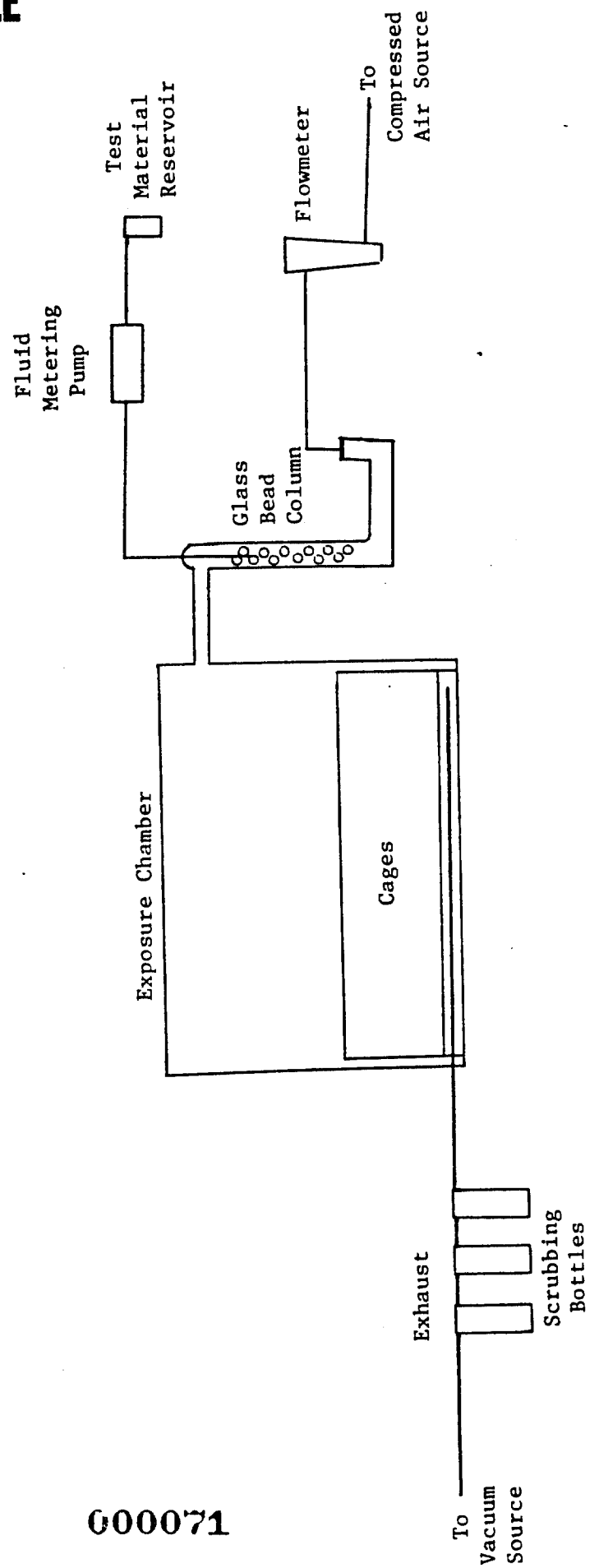

Figure 1. Vapor Generation and Exposure System
T-3607 Acute Inhalation Toxicity Test

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Compliance with TSCA Good Laboratory Practices

This study was conducted in accordance with current Toxic Substances Control Act (TSCA) Good Laboratory Practices with the following variation:

1. The Sponsor provided the purity of the test material and indicated that it would be stable for the duration of the study. However, the composition and other characteristics were not available for the testing facility. An identification or CAS Registry Number was not available from the Sponsor.

It is the opinion of the study director that these factors did not influence the results or interpretation of this study.

Donald J Nachreiner 12-11-84

D. J. Nachreiner, B.S. Date
Study Director

WPC/rkk/0500B-1
12-10-84

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Quality Assurance Unit Study Inspection Summary

Test Substance: T-3607

Study: Acute Inhalation Toxicity

Study Director: D. J. Nachreiner, B.S.

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
5-20-83	Standard Protocol	5-20-83	5-20-83
10-2-84	Standard Protocol Amendment	10-3-84	10-3-84
12-4 to 12-5-84	Final Data and Final Report	12-6-84	12-13-84

Linda J. Calisti 12/17/84

L. J. Calisti Date
Group Leader
Good Laboratory Practices/Quality Assurance

000073

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3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

REPORT OF ANALYSIS

W. C. MCCORMICK
MINNESOTA MINING AND MANUFACTURING
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 407039
DATE ENTERED: 07/19/
REPORT PRINTED: 10/05/

SAMPLE: T-3607

PURCHASE ORDER NUMBER: P-688709-405

ENCLOSED: ACUTE ORAL TOXICITY STUDY - METHOD, SUMMARY, PATHOLOGY
RAW DATA APPENDIX

SIGNED: Steven M. Glaza
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC. MADISON, WISCONSIN.

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

SAMPLE: T-3607

ACUTE ORAL TOXICITY

Test Animal: Young adult male and female albino rats (approximately 7 weeks of age) of the Sprague-Dawley strain were procured, maintained in group cages in temperature- and humidity-controlled quarters, provided continuous access to Purina Rodent Chow and water, and held for an acclimation period of at least 7 days.

Acclimated animals were chosen at random for the study. Test animals were housed by sex in groups of five and identified by animal number and corresponding ear tag. Food and water were available ad libitum throughout the study, except for an overnight period just before test material administration when food, but not water, was withheld.

Reason for Species Selection: The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Method: Five male and five female rats weighing between 202 and 231 g were used for a single dosage level of 5.0 g/kg of body weight.

Preparation and Administration of Test Material: An individual dose was calculated for each animal based upon its fasted body weight and administered undiluted by gavage. The dose volume was 2.76 ml/kg of body weight based upon the average bulk density of 1.81 g/ml.

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours following test material administration. The animals were observed daily thereafter for 14 days for clinical signs and twice daily for mortality.

All animals were weighed just before test material administration, at 7 days and at study termination.

Pathology: At study termination all animals were euthanatized, subjected to a gross necropsy examination and all abnormalities were recorded.

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SAMPLE NUMBER: 40703983

SAMPLE: T-3607

ACUTE ORAL TOXICITY (CONTINUED)

SUMMARY

Test Animal: Albino Rats - Sprague-Dawley strain
Source: Harlan Sprague-Dawley, Madison WI
Date Animals Received: 07/24/84

Method of Administration: Oral Gavage
Date Test Started: 08/03/84 Date Test Completed: 08/17/84

Estimated Oral LD50: Male - Greater than 5.0 g/kg of body weight
Female - Greater than 5.0 g/kg of body weight

Mortality Summary (Number of Deaths)

Dosage	Hours		Days															
Level	0 - 4		1		2		3		4		5		6		7-14		Total		
(g/kg)	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both
5.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	0/5	0/10

Average Body Weights (g)

	Initial	Day 7	Terminal
Male	221	276	295
Female	210	226	231

PATHOLOGY

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C21437	M	-	14	No Visible Lesions.
C21439	M	-	14	No Visible Lesions.
C21434	M	-	14	No Visible Lesions.
C21433	M	-	14	No Visible Lesions.
C21432	M	-	14	Lungs - multiple, raised, grey foc on all lobes, pinpoint to 2 mm in diameter.
C21521	F	-	14	No Visible Lesions.
C21503	F	-	14	No Visible Lesions.
C21522	F	-	14	No Visible Lesions.
C21523	F	-	14	No Visible Lesions.
C21524	F	-	14	No Visible Lesions.

000076

Reference: Hitch, R.K., "Acute Oral Toxicity Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substance Series 81-1, pp. 34-39 (November 1982).

GROSS CLINICAL OBSERVATIONS

Study Title: Acute Oral Toxicity

Test Article: T-3607 RT No. 10703983

Dosage Level: 5.0 g/kg Vehicle: N/A

Species: Rat Sex: ♂

Dose Time: 11:20 am

Number of Animals Affected/Observation Period

	Hours			Days													
Observations	10	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Technician	jm	jm	jm	el	el	dlb	jm	jm	dlb	jm	slh	slh	dlb	sg	dlb	dlb	sg
Date 1984	8/3	8/3	8/3	8/4	8/5	8/6	8/7	8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	8/16	8/17

NA = Not applicable
NE = Not evident

Reviewed by: JBW Date: 9-13-84

GROSS CLINICAL OBSERVATIONS

Study Title: Acute Oral Toxicity

Test Article: T-3607 RT No. 40703983

Dosage Level: 5.0 g/kg Vehicle: N/A

Species: Cat Sex: F

Dose Time: 11:30 am

Number of Animals Affected/Observation Period

	Hours			Days													
Observations	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Technician	jp	jp	jp	el	el	dlb	jp	jp	dlb	jp	dlb	dlb	dlb	SG	dlb	dlb	SG
Date 1984	8/3	8/3	8/3	8/4	8/5	8/6	8/7	8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	8/16	8/17

NA = Not applicable
NE = Not evident

Reviewed by: [signature] Date: 9-13-84

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3607 Vehicle NA RT No. 40703983

Bulk Density 1.81 (g/ml) Species Rat Source Harlan Date Received 7-24-84

Fasted: Date 8-2-84 Time 3:30pm Tech. dlh Room No. 3

Sex ♂

Dosage 5.0 (g/kg) / Dose Volume 2.76 (ml/kg)							Tech.	Date 1984	Scale Used:
	Dose Time 11:20 AM								
Animal No./Ear Tag No. C2	1437	1440	1439	1434	1433	1432	jp	8-3	NA
Prefasted Body Weight (g)	NA								→
Fasted Body Weight (g)	215	220	231	216	224	219	jp	8-3	Ktron 13019
Actual Dose (ml)	0.59	0.61	0.64	0.60	0.62	0.60	jp	8-3	NA
Day 7 Body Weight (g)	281	*	284	276	269	275	jp	8-10	Ktron 5228
Day 14 Body Weight (g)	300	*	308	289	288	291	SG	8/17	KTRON 15019
						Doses Verified by	slh	8-3	NA

Sex ♀

Dosage 5.0 (g/kg) / Dose Volume 2.76 (ml/kg)								Tech.	Date 1984	Scale Used:
	Dose Time 11:20 AM									
Animal No./Ear Tag No. C2	1521	1503	1520	~~1525~~ @	1522	1523	1524	jp	8/3	NA
Prefasted Body Weight (g)	NA									→
Fasted Body Weight (g)	218	218	239	~~206~~ @	206	208	202	jp	8/3	Ktron 13019
Actual Dose (ml)	0.60	0.60	0.66		0.57	0.57	0.56	jp	8/3	NA
Day 7 Body Weight (g)	229	232	*		223	224	220	jp	8/10	Ktron 5228
Day 14 Body Weight (g)	238	241	*		230	223	224	SG	8/17	KTRON 15019
						Doses Verified by	slh	8-3	NA	

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	Study Day 1		2		3		4		5		6		7		8		9		10		11		12		13		14		Total
		am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	
♂ 5.0 g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	0/5
♀ 5.0 g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	0/5
Technician	jp	dlh	dlh	jp	jp	jp	jp	jp	jp	dlh	dlh	jp	dlh	dlh	dlh	slh	dlh	slh	dlh	dlh	dlh	SG	dlh	dlh	SP	dlh	SP	SG	NA	slh
Date 1984	8/3	8/4	8/4	8/5	8/5	8/6	8/6	8/7	8/7	8/8	8/8	8/9	8/9	8/10	8/10	8/11	8/11	8/12	8/12	8/13	8/13	8/14	8/14	8/15	8/15	8/16	8/16	8/17	NA	9/13

NA - Not Applicable 0 entry error 8-3-84 jp

* - Dosage calculated, but not administered

Reviewed by JBW Date 9-13-84

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SRI International

IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2540 CoC and T-2541 CoC

Final Report

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SUMMARY

SRI International examined 3M Company compounds T-2540 CoC and T-2541 CoC for mutagenic activity with strains TA1535, TA1537, TA1538, TA98, and TA100 of Salmonella typhimurium in the standard Ames Salmonella/microsome assay, in an assay conducted in desiccators, and with the yeast Saccharomyces cerevisiae D3. Each assay was performed in the presence and in the absence of a rat liver metabolic activation system. Neither T-2540 CoC nor T-2541 Coc was mutagenic or recombinogenic in any of the assays performed.

INTRODUCTION

SRI International examined 3M Company compounds T-2540 CoC and T-2541 CoC for mutagenicity by in vitro microbiological assays with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium in the standard Ames Salmonella/microsome assay, in an assay conducted in desiccators, and with the yeast Saccharomyces cerevisiae D3. An Aroclor 1254-stimulated, rat liver homogenate metabolic activation system was included in the assay procedures to provide metabolic steps that the bacteria either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with S. typhimurium has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic.[1] The assay procedure with S. cerevisiae is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination.[2,3] However, because the assay systems do not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response conclusively proves that a chemical is hazardous or nonhazardous to man.

METHODS

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only cells that revert to histidine independence (his$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis. The his$^+$ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold, usually in a dose-related manner.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.[1,4-7] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio) and in the repair of ultraviolet (uv)-induced DNA damage (uvrb). The rfa mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB mutation causes decreased repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[7] In addition, plasmid pKM101 confers resistance to the

antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cell.[8] The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICI-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4°C on minimal agar plates supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains also contain ampicillin (25 µg/ml) to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every four to six weeks from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid, CM67).

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amino type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[6,9-11] Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.[9] In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyls). This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed with an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80°C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)
(4) 0.05 ml of a solution of the test chemical.

This mixture is stirred gently and then poured onto minimal agar plates.† After the top agar has set, the plates are incubated at 37°C for 3 days. The number of *his*$^+$ revertant colonies is counted and recorded.

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*The 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

†Minimal agar plates consist of, per liter, 15 g of agar, 10 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$.

For negative controls, we use steps (1), (2), and (3) (optional), and 0.05 ml of the solvent is used for the test chemical. Dimethylsulfoxide (DMSO) was used as the solvent for T-2540 CoC and T-2541 CoC. For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3), (optional), and (4).

Assays in Desiccators for Volatile Compounds

The standard Ames plate test is not entirely suitable for testing highly volatile chemicals, so we have modified the procedure to conduct such testing. The Salmonella plates are prepared as described for the assays in agar, but no test chemical is added. The plates, with lids removed, are placed side by side on a perforated shelf in a 9-liter desiccator (Figure 1). A known volume of the test chemical is added to a glass petri plate that is placed in the center of and attached to the bottom of the shelf. A control chemical is tested similarly in each experiment. The desiccator is sealed and placed on a magnetic stir plate in a room maintained at 37°C. A magnetic stirrer with vanes, placed in the base of each desiccator, ensures adequate dispersion of the chemical. After incubation for 8 hours, the plates are removed from the desiccators, their lids are replaced, and they are incubated at 37°C for an additional 64 hours. The number of his^+ revertants is counted and recorded.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.[2] When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various carcinogenic or recombinogenic agents. The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates.[3]

DESICCATOR ASSAY

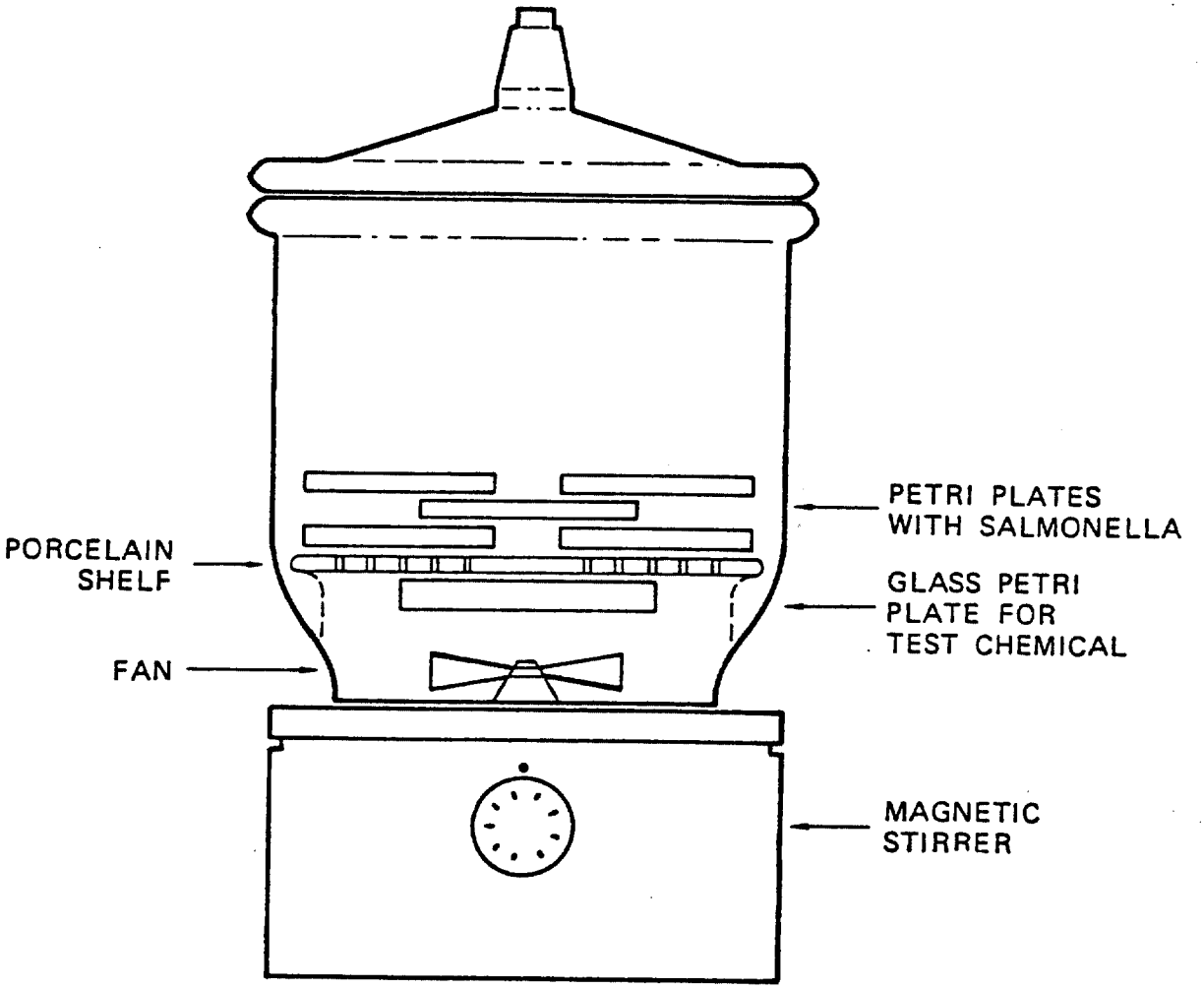

Figure 1

A stock culture of S. cerevisiae is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C with shaking.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged and the cells are resuspended at a concentration of 10^8 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.00 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of the test chemical
- 0.3 ml of buffer.

DMSO was used as the solvent for T-2540 CoC and T-2541 CoC. Several dose levels of the test chemical (up to 5%, w/v or v/v) are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline, and a volume of 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 2.0% agar; five plates are spread with the 10^{-3} dilution and three plates are spread with the 10^{-5} dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10 X magnification, and the number of mitotic recombinants (red colonies or red sectors) is recorded. The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than 3-fold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

RESULTS AND DISCUSSION

Tables 1 and 2 present the results of our tests of T-2540 CoC and T-2541 CoC in the Ames Salmonella/microsome assay. The data in each table show the results of a duplicate assay performed on separate days. Both compounds were tested over a wide range of dose levels, from 10 to 5,000 µg/plate, both with and without metabolic activation. Compound T-2540 CoC was toxic to the bacteria at 5,000 µg/plate. No dose-related increase in the number of histidine revertants over the background count was observed in either assay. Therefore, we conclude that compounds T-2540 CoC and T-2541 CoC were not mutagenic in the standard Salmonella plate incorporation assay.

Table 3 presents the results of an assay of T-2540 CoC and T-2541 CoC conducted in desiccators with strains TA98 and TA100. The compounds were tested over a range of dose levels from 0.1 to 5.0 ml per desiccator. Toxicity was observed at 1.0 ml per desiccator with T-2540 CoC and at 5.0 ml per desiccator with T-2541 CoC. Because no mutagenic response was observed with or without metabolic activation, no further testing was performed in desiccators.

The results of microbiological assays with S. cerevisiae D3 on T-2540 CoC are presented in Tables 4 through 6. In a preliminary experiment conducted over a range of concentrations from 0.1 to 5.0% (Table 4), organisms exposed to T-2540 CoC showed less than 50% survival at concentrations of 0.5% and higher. Therefore, the compound was retested over a range of concentrations from 0.025 to 0.25% (Table 5). There was a slight increase in the number of mitotic recombinants both with and without metabolic activation at concentrations from 0.075 to 0.25%, and this increase was not dose-related. Compound T-2540 CoC was tested once more within a narrower range, from 0.07 to 0.2% without activation and from 0.09 to 0.4% with activation (Table 6). In this assay, there was an increase in the number of mitotic recombinants only

at 0.3% with activation. Because the increases in mitotic recombinants observed in both assays were neither dose-related nor reproducible, we do not believe that T-2540 CoC was recombinogenic with S. cerevisiae D3.

Tables 7 through 9 present the results of assays with T-2541 CoC with S. cerevisiae D3. A preliminary experiment determined that T-2541 CoC was toxic to the yeast at 0.5% without activation and 5.0% with activation (Table 7). The compound was tested twice more at concentrations from 0.025 to 0.25% without activation and 0.25 to 2.5% with activation (Table 8), and at concentrations from 0.07 to 0.2% without activation and 0.2 to 1.0% with activation (Table 9). T-2541 CoC showed several increases in the number of mitotic recombinants per 10^5 survivors. However, as with T-2540 CoC, these increases were neither dose-related nor repeatable; therefore, we conclude that T-2541 CoC was not recombinogenic in this assay.

In summary, we conclude that compound T-2540 CoC and T-2541 CoC were not mutagenic with S. typhimurium, or recombinogenic with S. cerevisiae D3.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative control	-		27	24	9	12	28	18	31	43	125	112
(DMSO)	+		28	28	43	48	50	41	53	43	110	136
Positive control												
Sodium azide	-	1.0	569	531							836	859
9-Aminoacridine	-	50.0			576	261						
2-Nitrofluorene	-	5.0					727	537	371	387		
2-Anthramine	-	1.0					17	20	20	31	136	102
	+	1.0					251	306	268	219	373	417
	-	2.5	21	26	18	7						
	+	2.5	182	194	81	67						
T-2540 CoC	-	10	26	19	12	8	18	20	40	21	124	103
	-	50	19	37	7	13	16	15	25	37	98	88
	-	100	19	24	8	7	14	14	27	27	101	102
	-	500	16	27	4	8	16	19	26	39	117	100
	-	1,000	32	18	19	9	18	17	32	42	111	100
	-	5,000	19T*	19T	6T	5T	15T	12T	16T	30	85T	86T
	+	10	33	25	36	29	28	28	51	42	105	99
	+	50	31	37	33	39	24	29	42	48	108	115
	+	100	24	29	40	26	26	32	33	48	134	126
	+	500	30	31	18	30	26	33	33	39	100	125
	+	1,000	21	39	30	27	36	30	39	29	97	126
	+	5,000	26	28	18	19	38T	25T	44	43	104T	93T

(Continued)

Table 1 (Concluded)

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
T-2541 CoC	-	10	27	31	12	7	26	17	24	26	113	105
	-	50	20	28	4	5	15	15	43	25	115	127
	-	100	33	29	6	6	12	6	30	38	90	100
	-	500	19	28	17	6	12	9	32	27	123	97
	-	1,000†	32	25	17	20	12	21	33	31	105	102
	-	5,000†	29	25	13	13	15	17	33	27	109	112
	+	10	25	18	21	30	29	17	38	50	98	110
	+	50	29	17	19	36	32	25	44	38	114	109
	+	100	28	27	28	26	19	29	45	43	126	103
	+	500	20	28	30	30	15	21	48	44	142	135
	+	1,000†	38	29	32	26	37	30	32	50	135	112
	+	5,000†	30	46	27	24	26	30	51	47	109	111

*T, toxic.

†The compound formed a precipitate at this concentration.

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Control	-		21	20	5	10	16	15	17	19	109	102
DMSO	+		7	21	28	9	13	28	32	26	105	103
Positive Control												
Sodium azide	-	1.0	505	380							624	667
9-Aminoacridine	-	50.0			442	390						
2-Nitrofluorene	-	5.0					708	678	383	386		
2-Anthramine	-	1.0					21	20	25	25	116	101
	+	1.0					420	258	126	141	576	430
	-	2.5	19	24	5	6						
	+	2.5	221	256	131	158						
T-2540 CoC	-	10	20	38	6	8	9	17	26	32	90	125
	-	50	25	28	7	14	19	14	25	20	92	87
	-	100	33	17	7	14	16	20	20	16	109	92
	-	500	29	20	12	3	8	9	19	28	93	101
	-	1,000	21	16	12	5	13	7	24	27	88	108
	-	5,000	T*	6T	T	1T	9T	5T	14T	20T	67T	67T
	+	10	17	13	21	13	15	17	31	27	96	88
	+	50	19	16	22	14	14	15	29	29	98	108
	+	100	10	19	25	15	17	18	26	43	89	98
	+	500	16	4	14	12	7	20	27	31	76	97
	+	1,000	18	5	19	15	14	23	28	29	93	99
	+	5,000	14	15	14	14	18	16	14	21	81	33T

(Continued)

Table 2 (Concluded)

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
T-2541 CoC	-	10	29	21	8	8	9	13	28	17	109	96
	-	50	26	28	4	7	6	8	13	24	108	99
	-	100	34	24	13	7	13	9	29	26	101	113
	-	500	21	28	8	9	8	8	19	27	90	101
	-	1,000†	20	26	13	13	13	7	17	21	99	111
	-	5,000†	15	19	9	14	12	7	26	20	104	103
	+	10	7	15	13	9	26	17	20	32	85	96
	+	50	12	15	11	7	17	15	24	25	92	97
	+	100	18	15	8	6	25	21	24	18	101	87
	+	500	9	8	14	8	12	24	27	29	101	91
	+	1,000†	14	14	18	27	24	17	32	19	98	109
	+	5,000†	13	14	12	18	19	24	19	31	110	103

*T, toxic

†The compound formed a precipitate at this concentration.

Table 3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM IN DESICCATORS
8-HOUR EXPOSURE
T-2540 CoC and T-2541 CoC

Compound	Metabolic Activation	Milliliters of Compound in Desiccator	Histidine Revertants per Plate			
			TA98		TA100	
Negative Control DMSO	-		16	30	160	131
	+		33	51	142	161
Positive Control Methylene chloride	-	1.0	757	847	1478	1725
	+	1.0	817	807	1513	1476
T-2540 CoC	-	0.1	26	29	138	152
	-	0.5	28	14	99	110
	-	1.0	12T*	25T	T	5T
	-	5.0	T	T	T	T
	+	0.1	28	38	123	135
	+	0.5	52	52	139	158
	+	1.0	16T	9T	17T	15T
	+	5.0	T	T	T	T
T-2541 CoC	-	0.1	37	27	125	140
	-	0.5	24	28	97	128
	-	1.0	21	29	109	141
	-	5.0	19	13T	16T	51T
	+	0.1	36	46	138	111
	+	0.5	41	31	149	148
	+	1.0	28	29	124	127
	+	5.0	29	29	99T	103T

*T, toxic.

Table 4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		6.5	100	3.5	5.4
DMSO	+		7.2	100	3.5	4.9
Positive Control	-	0.025	2.5	38	608	2400
1,2,3,4 Diepoxybutane	+	0.025	5.8	81	928	1600
T-2540 CoC	-	0.1	5.6	86	5.0	8.9
	-	0.5	2.3	35	2.0	8.7
	-	1.0	1.5	23	3.0	20
	-	5.0	T*	T	T	T
	+	0.1	7.7	107	6.0	7.8
	+	0.5	3.5	49	4.0	11
	+	1.0	1.1	15	3.0	27
	+	5.0	T	T	T	T

*T, toxic.

Table 5

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		7.2	100	2.0	2.8
DMSO	+		7.6	100	2.5	3.3
Positive Control	-	0.025	3.6	50	923	2600
1,2,3,4 Diepoxybutane	+	0.025	7.0	92	1040	1500
T-2540 CoC	-	0.025	6.8	94	4.0	5.9
	-	0.05	6.5	90	7.0	11
	-	0.075	5.8	81	11.0	19
	-	0.1	5.7	79	6.0	11
	-	0.25	2.7	38	10.0	37
	+	0.025	7.4	97	8.0	11
	+	0.05	7.0	92	3.0	4.3
	+	0.075	5.2	68	8.0	15
	+	0.1	5.1	67	6.0	12
	+	0.25	5.3	70	14.0	26

Table 6

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2540 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		8.3	100	3.0	3.6
DMSO	+		7.6	100	4.0	5.3
Positive Control	-	0.025	5.2	63	880	1700
1,2,3,4 Diepoxybutane	+	0.025	6.3	83	680	1100
T-2540 CoC	-	0.07	7.8	94	6.0	7.7
	-	0.08	8.2	99	3.0	3.7
	-	0.09	6.0	72	5.0	8.3
	-	0.1	5.7	69	3.0	5.3
	-	0.2	4.7	57	7.0	15
	+	0.09	9.3	122	5.0	5.4
	+	0.1	6.1	80	5.0	8.2
	+	0.2	5.8	76	4.0	6.9
	+	0.3	3.2	42	10	31
	+	0.4	3.0	39	3.0	10

Table 7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		6.5	100	3.5	5.4
DMSO	+		7.2	100	3.5	4.9
Positive Control	-	0.025	2.5	38	608	2400
1,2,3,4 Diepoxybutane	+	0.025	5.8	81	928	1600
T-2541 CoC	-	0.1	5.0	77	1.0	2.0
	-	0.5	2.3	35	4.0	17
	-	1.0	T*	T	T	T
	-	5.0	T	T	T	T
	+	0.1	7.0	97	4.0	5.7
	+	0.5	5.6	78	2.0	3.6
	+	1.0	5.1	71	4.0	7.8
	+	5.0	2.1	29	6.0	29

*T, toxic.

Table 8

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		7.2	100	2.0	2.8
DMSO	+		7.6	100	2.5	3.3
Positive Control	-	0.025	3.6	50	923	2600
1,2,3,4 Diepoxybutane	+	0.025	7.0	92	1040	1500
T-2541 CoC	-	0.025	5.5	76	6.0	11
	-	0.05	5.5	76	3.0	5.5
	-	0.075	5.1	71	3.0	5.9
	-	0.1	5.7	79	5.0	8.8
	-	0.25	2.7	38	7.0	26
	+	0.25	5.5	72	8.0	15
	+	0.5	5.1	67	7.0	14
	+	0.75	3.6	47	2.0	5.6
	+	1.0	3.1	41	7.0	23
	+	2.5	T*	T	T	T

*T, toxic.

Table 9

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
T-2541 CoC

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Control	-		8.3	100	3.0	3.6
DMSO	+		7.6	100	4.0	5.3
Positive Control	-	0.025	5.2	63	880	1700
1,2,3,4-Diepoxybutane	+	0.025	6.3	83	680	1100
T-2541 CoC	-	0.07	8.2	99	5.0	6.1
	-	0.08	7.7	93	3.0	3.9
	-	0.09	6.0	72	6.0	10
	-	0.1	6.4	77	8.0	13
	-	0.2	5.2	63	6.0	12
	+	0.2	6.5	86	4.0	6.2
	+	0.4	8.7	114	5.0	5.8
	+	0.6	6.2	82	6.0	9.7
	+	0.8	5.4	71	11	20
	+	1.0	4.4	58	5.0	11

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3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-7098.1
PHARMACOKINETIC STUDY OF POSF IN RATS

***Study Objective*:**
The objective of this study is to assess the potential for oral absorption, urinary and fecal clearance and biological persistence of perfluorooctane sulfonyl fluoride (POSF) in male Sprague Dawley rats after a single oral dose. The POSF compound is the starting material for the synthesis of a wide variety of perfluorooctane sulfonate (PFOS) based materials. The purpose of this study is to understand the rate of metabolism of POSF to PFOS by the liver. This study will provide data for proper risk characterization of POSF.

Research Client: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55133-3220

Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55133-3220

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55133-3220

Study Director: Andrew M. Seacat, Ph.D.
Sr. Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph.: 651-575-3161 FAX: 651-733-1773

***Study Toxicologist*:** Deanna Nabbefeld, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline (Assuming EHS&R approval on Jan. 5th.)*:*
In-Life Start Date: January 11th, 1999
In-Life End Date: February 9th, 1999
Analytical Completion Date: March 22nd, 1999
Final Report Completion Date: April 19th, 1999

Regulatory Compliance:

This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:

Dan Hakes, Product Responsibility Liaison 3M Chemicals Division, will furnish high-purity POSF.

Identification:

Name: Perfluorooctane Sulfonyl Fluoride

Molecular Formula: To be provided.

Lot Number: The lot numbers will be maintained in the raw data.

Purity:

Documentation will be kept in on file.

Stability:

Documentation will be kept on file.

Storage Conditions:

Upon receipt, test material will be stored tightly sealed at room temperature.

Characteristics:

Information on synthesis methods, composition or other characteristics that define the test material will be kept on file.

Animals:

Species: Rat
Strain: Sprague Dawley
Source: Harlan
Age at initiation of treatment: 6-8 weeks
Weight at initiation of treatment: approximately 150-250g
Number and sex: 30 males

Table 1 - Dose Groups

Group	Dose	N	Euthanasia
*1	0 mg/kg	5	day 1 post dose
*2	0 mg/kg	5	day 4 post dose
*3	0 mg/kg	5	day 29 post dose
4	5 mg/kg	5	day 1 post dose
5	5 mg/kg	5	day 4 post dose
6	5 mg/kg	5	day 29 post dose

** Rats in groups 1-3 will be used concomitantly as control animals in studies T-7071.2, Pharmacokinetic Study of M556 in Rats, and T-7099.1, Pharmacokinetic Study of FX-845 in Rats.*

Identification: ear tag with animal number or unique tail mark.
AUA Number: 2154

Husbandry:

Housing:

Three specific rats from groups 3 and 6 will be housed individually in metabolism cages for portions of the study (see Table 2). When not in metabolism cages, these rats will be group housed in standard cages. All other rats will be group housed in standard cages throughout the study.

Diet/Water:

Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, supplied by Harlan Teklad, Madison, WI, and tap water will be provided to all rats *ad libitum* throughout the study.

Environment:

Environmental controls for the animal room will be set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose and Dosing Procedures:

Method of administration/Dose preparation:

A single 5mg/kg dose of POSF will be administered via oral gavage to rats in groups 4-6 on day zero of the study. The POSF will be prepared as a 1% (1 mg/ml) uniform suspension in 2% Tween 80 using a 15 ml tissue grinder. A volume of 5 ml suspension / kg body weight will be administered to each rat. Re-suspension of solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing.

A single 5 ml / kg body weight dose of 2% Tween 80 will be administered via oral gavage to rats in groups 1-3 on day zero of the study.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily for mortality and morbidity and notable findings will be recorded. Additional findings will be recorded as they are observed.

Body Weights:

Each animal will be weighed immediately prior to dosing, weekly thereafter and immediately prior to euthanasia.

Specimen Collection:

Frequency (see Table 2):

Urine and feces collections will be made on days 1, 2, 4, 14 and 29 post dose. Necropsies will be performed on days 1, 4 and 29 post dose.

Table 2 - Schedule

Jan 10	**Jan 11** **day 0** DOSING	**Jan 12** **day 1 PD** Collection Dy 1 PD sac	**Jan 13** **day 2 PD** Collection Switch to reg cages.	**Jan 14** **day 3 PD** Switch to met cages.	**Jan 15** **day 4 PD** Collection Switch to reg cages. Dy 4 PD sac.	**Jan 16** **day 5 PD**
Jan 17 **day 6 PD**	**Jan 18** **day 7 PD**	**Jan 19** **day 8 PD**	**Jan 20** **day 9 PD**	**Jan 21** **day 10 PD**	**Jan 22** **day 11 PD**	**Jan 23** **day 12 PD**
Jan 24 **day 13 PD**	**Jan 25** **day 14 PD** Switch to met cages.	**Jan 26** **day 15 PD** Collection Switch to reg. cages.	**Jan 27** **day 16 PD**	**Jan 28** **day 17 PD**	**Jan 29** **day 18 PD**	**Jan 30** **day 19 PD**
Jan 31 **day 20 PD**	**Feb 1** **day 21 PD**	**Feb 2** **day 22 PD**	**Feb 3** **day 23 PD**	**Feb 4** **day 24 PD**	**Feb 5** **day 25 PD**	**Feb 6** **day 26 PD**
Feb 7 **day 27 PD**	**Feb 8** **day 28 PD** switch to met cages	**Feb 9** **day 29 PD** collection Dy 29 PD sac				

Method of Specimen Collection:

Urine and feces will be collected from each metabolism cage at the designated times. The initial volume of urine will be recorded, the sides of the urine collection apparatus will be washed with 10-20 ml deionized water and the final volume of urine will be brought to 45 ml with additional deionized water. Daily feces weight will be recorded for each animal. At the designated times, animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, blood (≈ 6 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. Blood samples will be allowed to clot for a period of 15 to 30 minutes at room temperature, and the clot will be spun down in a centrifuge at 1100 x g for 5 minutes. The serum will be transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. Each sera sample will then be transferred to a separate labeled polypropylene microfuge tube and flash-frozen in liquid nitrogen. Liver, kidneys and subcutaneous fat from each animal will be removed, weighed, flash frozen in liquid nitrogen and placed individually into labeled sterile sample bags. The remainder of each carcass will be placed in a labeled ziplock bag and frozen (-70 °C) until analysis.

Specimen Handling:

Specimens will temporarily be stored in a freezer set to maintain -60 to -80°C. For metabolite analysis, these specimens will be packed in dry ice and shipped to:

Kris Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
St. Paul, MN 55133-3331
Ph: 612-778-6081, FAX: 612-778-6176.

The 3M Environmental Laboratory will manage the extraction and analysis of sera, liver, urine and feces for the parent compound, POSF, and its presumed metabolite, PFOS. All tissue samples will be retained for possible future analysis of total organic fluorine (TOF) if deemed necessary by the Study Director. 3M Environmental Laboratory or its designee would perform this analysis. All results will be provided for inclusion in the final report.

The number, type and date of collection of specimens to be generated for analysis are as follows:

Table 3 - Specimens

Specimens	day 1 post dose	day 2 post dose	day 4 post dose	day 15 post dose	day 29 post dose	Total
Serum	10		10		10	30
Liver	10		10		10	30
Kidneys	10		10		10	30
Subcutaneous fat	10		10		10	30
Carcass	10		10		10	30
Urine	6	6	6	6	6	30
Feces	6	6	6	6	6	30

Data Analysis:
Data collected on tissue levels of parent compound and identifiable metabolites will be analyzed for toxicokinetic parameters and for statistically significant differences between groups using Students T-test and/or ANOVA.

Responsibilities:
- Deanna Nabbefeld and Andrew Seacat will be responsible for dosing the animals, collecting in-life specimens, performing the necropsy and collecting and sending tissue samples for analysis.
- Kris Hansen, 3M Environmental, will be responsible for analytical analysis.
- Andrew Seacat will draft a final report and ensure the report receives appropriate 3M review before a final report is issued.

Signatures:

Andrew Seacat 1/8/99

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna Nabbefeld 1/8/99

Deanna Nabbefeld, MS
Advanced Toxicologist
Study Toxicologist

Date

Sponsor Representative

Date

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

2. FOSA	Perfluorooctanesulfonamide

Acute Toxicity

1) Acute Oral Toxicity Screen with T-3421 in Albino Rats, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883AR0287, 3M Reference No. T-3421 (KTZ-15), January 17, 1984

2) Acute Ocular Irritation Test with T-3421 in Albino Rabbits, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883EB286, 3M Reference No. T-3421 (KTZ-15), August 24, 1983

3) Primary Skin Irritation Test with T-3421 in Albino Rabbits, Safety Evaluation Laboratory, Riker laboratories, Inc., Project No. 0883AR0288, 3M Reference No. T-3421 (KTZ-15), August 9, 1983

Studies in Progress

1) Protocol, Feces Method Development Metabolism Study for Perfluorooctanesulfonate Derivatives [N-EtFOSE, PFOS, and FOSA], 3M Strategic Toxicology Laboratory, Study Nos., T-636.17; T-6295.21; T-7132.3; ST-41, In-Life Start Date November 22, 1999, In-Life End Date November 24, 1999

2) Protocol, Pharmacokinetic Study of Perfluorooctane Sulfonamide [FOSA] in Rats, 3M Strategic Toxicology Laboratory, Study Nos., T132.2; ST-39, In-Life Start Date October 4, 1999, In-Life End Date November 2, 1999

3) Protocol, Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluorooctanesulfonamide (PFOSA 3M T-7091.1) in Rats, Pathology Associates International, Study No. 1132-100

Acute Oral Toxicity Screen with T-3421 in Albino Rats

Experiment No.: 0883AR0287

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: August 2, 1983 to September 7, 1983

Conducted By:
D. M. Markoe, Jr., BS — Date 12/30/83
Toxicologist
Study Director

Reviewed By:
K. D. O'Malley, BS — Date 1/12/84
Senior Toxicologist
Acute Toxicology

K. L. Ebbens, BS — Date 1/17/84
Supervisor, Toxicology Testing

dc:
M. T. Case
D. D. Griffith
W. C. McCormick

Summary

The acute oral toxicity screen with T-3421 was conducted from August 2, 1983 to September 7, 1983 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female albino rats ranging in body weight from 191-258 grams. The test article was administered by gastric intubation at dosage levels of 5,000, 2,000, 500 and 200 mg/kg body weight with mortalities of 10/10, 10/10, 10/10 and 2/10 noted respectively from one hour to six days post dose administration. The untoward behavioral reactions which occurred during the 14 day observation period generally consisted of hypoactivity, lethargy, prostration, diarrhea and unkempt appearance with the onset occurring from 1-30 minutes to day four post dose administration. Clonic convulsions were noted in two animals prior to death, trancient alopecia was noted in two animals while dyspnea and salivation were noted in one animal during the study. All reactions subsided by day eleven or death precluded recovery. Body weight gains were noted in animals which survived the study period. Necropsies performed at termination of the study revealed no visible lesions while hyperemic or hemorrhagic lungs and/or hemorrhagic intestinal tract generally were noted in the animals which died during the conduct of the study. The acute oral LD50 of T-3421 is greater than 200 mg/kg and less than 500 mg/kg in male and female albino rats.

Introduction

The objective of this study was to determine the acute oral LD50 of T-3421 in albino rats. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rats[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rats were housed in stock cages in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] plus water *ad libitum* except during the 16 hour period immediately prior to gastric intubation when food was withheld.

Five male and five female rats were administered the test material at preselected dosage levels. The doses were administered at a constant volume of 10 ml/kg directly into the stomachs of the rats using a hypodermic syringe equipped with an intubation needle.

After gastric administration of the test material, the rats were returned to their cages and observed for the following 14 days. Initial, seven day and final body weights, mortalities (Table 1) and adverse reactions (Table 2) were recorded. A necropsy was conducted on all animals that died during the study as well as those euthanatized at the end of the 14 day observation period (Table 1). The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

[a] King Labs, Oregon, WI
[b] Ralston Purina Laboratory Chow, Ralston Purina, St. Louis, MO

TABLE 1
ACUTE ORAL TOXICITY STUDY - ALBINO RATS
with T-3421
Mortality, Necropsy and Body Weight Data

Dose [a] (mg/kg)	Sex	Animal Number	Individual Body Weights (g) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
5000	M	3R3553	208	(Day 6)	-	5/5	100
		3R3554	210	(Day 2)	-		
		3R3555	197	(Day 6)	-		
		3R3556	219	(1 Hour)	-		
		3R3557	214	(Day 6)	-		
5000	F	3R3573	191	(1 Hour)	-	5/5	100
		3R3574	207	(1 Hour)	-		
		3R3575	207	(1 Hour)	-		
		3R3576	192	(1 Hour)	-		
		3R3577	209	(Day 1)	-		
2000	M	3R3558	195	(Day 6)	-	5/5	100
		3R3559	207	(Day 6)	-		
		3R3560	209	(Day 6)	-		
		3R3561	217	(Day 6)	-		
		3R3562	207	(Day 2)	-		
2000	F	3R3578	199	(Day 6)	-	5/5	100
		3R3579	218	(1 Hour)	-		
		3R3580	201	(1 Hour)	-		
		3R3581	205	(Day 1)	-		
		3R3582	203	(1 Hour)	-		
500	M	3R4070	210	(Day 3)	-	5/5	100
		3R4071	212	(Day 4)	-		
		3R4072	213	(Day 6)	-		
		3R4073	217	(Day 5)	-		
		3R4074	216	(Day 4)	-		
500	F	3R4106	204	(Day 6)	-	5/5	100
		3R4107	200	(Day 4)	-		
		3R4108	205	(Day 5)	-		
		3R4109	221	(Day 3)	-		
		3R4110	198	(Day 4)	-		

TABLE 1 (concluded)

ACUTE ORAL TOXICITY STUDY - ALBINO RATS

with T-3421

Mortality, Necropsy and Body Weight Data

Dose [a] (mg/kg)	Sex	Animal Number	Individual Body Weights (g) Test Day Number: 0	7	14	Number Dead / Number Tested	Percent Dead
200	M	3R4075	251	(Day 6)	-	1/5	20
		3R4076	236	274	297		
		3R4077	253	303	336		
		3R4078	258	302	345		
		3R4079	248	249	297		
200	F	3R4111	220	230	243	1/5	20
		3R4112	212	242	242		
		3R4113	217	236	236		
		3R4114	238	253	257		
		3R4115	225	(Day 5)	-		

Note: Figures in parenthesis indicate time of death

[a] Test article administered as a suspension in water.
The approximate oral LD50 is greater than 200 mg/kg and less than 500 mg/kg in fasted male and female albino rats.

Necropsy

Necropsy of the animals which survived the observation period revealed no visible lesions while necropsy of those animals which died during the conduct of the study generally had hyperemic or hemorrhagic lungs. Hemorrhagic small intestine was noted at the 500 mg/kg dose level and one animal from the 200 mg/kg dose group. One incidence of mottled liver was noted at the 5,000 mg/kg level.

TABLE 2

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3421

Summary of Reactions

Dose mg/kg	Reactions / Sex	Observation Periods: Number Affected/Number Dosed — Minutes: 1-30	60	120	Days: 1	2	3	4	5	6	7	8	9	10	11	12	13	14
5000	M																	
	Hypoactivity		2/5	0/4		3/3	3/3	-	-	*								
	Lethargy	2/5	3/5	4/4	4/4	0/3												
	Salivation	1/5	0/5															
	Dyspnea		1/5	0/4														
	Diarrhea				4/4	3/3	3/3	-	-	*								
	Unkempt Appearance					3/3	3/3	-	-	*								
5000	F																	
	Hypoactivity	1/5	0/3															
	Lethargy	4/5	1/3	0/1														
	Prostration		2/3	1/1	*													
2000	M																	
	Hypoactivity		2/5	2/5	5/5	4/4	4/4	-	-	*								
	Lethargy	3/5	3/5	1/5	0/5													
	Prostration			2/5	0/5													
	Diarrhea				5/5	4/4	4/4	-	-	*								
	Unkempt Appearance					4/4	4/4	-	-	*								
2000	F																	
	Hypoactivity	2/5	1/2	0/2	1/1	1/1	1/1	-	-	*								
	Lethargy	3/5	1/2	0/2														
	Prostration			2/2	0/1													
	Diarrhea				1/1	1/1	1/1	-	-	*								
	Unkempt Appearance					1/1	1/1	-	-	*								

TABLE 2 (concluded)

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3421

Summary of Reactions

Dose mg/kg	Reactions / Sex	Observation Periods: Number Affected/Number Dosed — Minutes 1-30	60	120	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
500	M																	
	Hypoactivity							2/3	1/1	*								
	Convulsions (clonic)							1/3	0/1									
500	Hypoactivity							3/3	2/2	*								
	Ataxia						1/4	0/3										
200	M																	
	Convulsions (clonic)								1/5	0/4								
200	F																	
	Alopecia											2/4	2/4	—	2/4	0/4		

Key:
Blank indicates no significant reactions
- observations inadvertenly missed over weekend
* Total Death

Riker Experiment No.: 83-3477-7

APPENDIX I

PROTOCOL

TEST: Acute Oral Toxicity Scan

SPONSOR: 3M Commercial Chemical Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-7471

CONTROL ARTICLE: none

PROPOSED STARTING/COMPLETION DATE OF TEST: 6/83 - 9/83

TEST SYSTEM: ALBINO RAT SD

SOURCE: KING LABS, OREGON, WI

Sex: M,F
Number: 5 5
Weight Range: 250 - 300 gm

OBJECTIVE: The objective of this test will be to characterize the acute oral toxicity of the test article in albino rats. Rats were selected as a test system for reproducibility of response, historical use, ease in handling and general availability.

METHOD: The animals will be housed in stainless steel suspended wire mesh cages in temperature and humidity controlled rooms during both the quarantine and test periods, with food[a] and water offered *ad libitum*[b]. Each animal will be identified by color coding, according to the laboratory's standard operating procedure, which will correspond to the animal numbers on a card affixed to the outside of the cage. A single dosage of 5,000 mg/kg will be administered each animal, however, if this dosage level does not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, the animals will be returned to their cages and observed for any untoward behavioral reactions for the following 14 days. Initial and final body weights will be recorded. A gross necropsy which will include, but not be limited to heart, lungs, liver, kidneys and general gastrointestinal tract will be conducted on all animals which die during the conduct of the test as well as the animals surviving the test period. Any gross abnormalities which are observed during the conduct of the necropsy will be recorded with specific mention to the organ and/or site observed. The acute medial lethal dose (LD_{50}) of the test article will be calculated, if possible, using a probit analysis method at the end of the observation period. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri
[b] FOOD WILL BE WITHHELD FOR A 16-20 HOUR PERIOD PRIOR TO DOSING.

W. C. McCormick 6-7-83
Sponsor Date

Study Director Date

APPENDIX I (concluded)
Deviations and/or Amendments to Protocol

1. Weekend observation for August 6 and 7 were inadvertently missed and on September 3rd.

D. M. Markoe, Jr. — Study Director — 10/5/83 Date

2. Due to a delay in study conduct the proposed completion date should be amended to 1/84.

D. M. Markoe, Jr. — Study Director — 12/29/83 Date

3. _____

Study Director — Date

4. _____

Study Director — Date

5. _____

Study Director — Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
D. M. Markoe, Jr., BS	Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

KTZ-15 (CC#341-4)
T-3421

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 25 May 83 J. Hoffinder

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes V no ____

3. The stability of the test and/or control substances have been determined or will be determined as of 25 Jun 83 or end of Test

The above information and documentation are located in the sponsor's records.

D. Parker 5/25/83
Sponsor Date

cc L. D. Winder 236-2B
W. C. McCormick 220-2E
W. H. Pearlman 223-65E
D. Party 236-1

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0783AR6227

This short term study was audited by Compliance Audit, and the final report examined against the raw data on January 25, 1984. The results of the audit were reported to the study director and to management on January 25, 1984.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

Carl E. Van Buskirk
Compliance Audit

January 25, 1984
Date

Acute Ocular Irritation Test

with T-3421

in Albino Rabbits

Experiment No.: 0883EB0286

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: June 21, 1983 to August 1, 1983

Conducted By: 8/15/83
D. M. Markoe, Jr., BS Date
Toxicologist
Study Director

Reviewed By: 8/23/83
K. D. O'Malley, BS Date
Senior Toxicologist
Acute Toxicology

8/24/83
K. L. Ebbens, BS Date
Supervisor, Toxicology Testing

dc: M. T. Case
F. D. Griffith
[redacted]

Summary

The results of the acute ocular irritation test conducted from June 21, 1983 to August 1, 1983 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3421 is moderately irritating (40.0/110.0) to the unwashed eye of the female albino rabbit. A five second and thirty second contact washed eye procedure were also conducted employing a five liter wash. The irritation ratings for T-3421, using the limited contact procedures, were mildly irritating (15.7/110.0) for the five second and mildly irritating (18.3/110.0) for the thirty second contact procedure.

Minimal corneal opacity, iritis and moderate conjunctivitis were produced during the unlimited contact procedure by the one hour evaluation. The irritation subsided to minimal corneal opacity with vascularization (two animals) and slight conjunctivitis (three animals) by the day seven evaluation.

T-3421, when allowed a five and thirty second contact, produced iritis and mild conjunctivitis by the one hour evaluation. All irritation subsided by the day two evaluation. Corneal opacity was not observed in any animal of either treatment group.

Introduction

The objective of this study was to assess the acute ocular irritation properties of T-3421 when instilled into the washed and unwashed eye of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits of the New Zealand breed[a] were used to evaluate the ocular irritating properties of the test article. The test method was modeled after that of Draize et al[b].

The test article was instilled into the conjunctival sac of the right eye of each rabbit according to the treatment procedure presented in Table 1 with the left eye of each animal serving as a control. At each scoring interval, the cornea, iris and palpebral conjunctiva were examined and graded for irritation and injury according to a standard scoring system[b]. The maximum possible score at any one examination and scoring period 110 points, which indicates maximal irritation and damage to all three ocular tissues (cornea, iris, conjunctiva) while a score of zero indicates no irritation (Table 2). In this scoring system, special emphasis is placed upon irritation or damage to the cornea, while less emphasis is placed upon damage to the iris and conjunctiva.

After completion of the test, the scores were analyzed, and a descriptive eye irritation rating was assigned to the test article. The criteria used for assignment of the descriptive rating were the frequency, the extent and the persistence of irritation or damage which occurred to the three ocular tissues (Table 3). The individual results are presented in Tables 4-6.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

The rating is arrived at by selecting the maximum mean irritation score at one hour, one, two or three days after instillation. If the rate of dissipation of injury does not meet the requirements defined for the descriptive rating appropriate for a particular numerical score, the descriptive rating is raised by one or more levels. The rating system is presented in Table 3. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Eye Irritation Test - Albino Rabbits

Treatment Procedure

Test Article	Number of Animals Evaluated	Form Administered	Quantity of Test Article Administered	Contact Period (seconds)	Volume of Wash (tap water)	Evaluation Time Post Dose Administration
T-3421	6	waxy solid	0.1 gm	unlimited	none	1 Hour, 1, 2, 3 and 7 Days
T-3421	3	waxy solid	0.1 gm	5 seconds	none	1 Hour, 1, 2, 3 and 7 Days
T-3421	3	waxy solid	0.1 gm	30 seconds	none	1 Hour, 1, 2, 3 and 7 Days

Table 2

Eye Irritation Test - Albino Rabbits

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Draize Grade
Conjunctiva	Redness (A)	
	Redness (refers to palpebral conjunctiva only). Vessels definitely injected above normal.	1
	More diffuse, deeper crimson red, individual vessels not easily discernible.	2
	Diffuse beefy red.	3
	Chemosis (B)	
	Any swelling above normal (included nictitating membrane).	1
	Obvious swelling with partial eversion of the lids.	2
	Swelling with lids about half-closed.	3
	Swelling with lids about half-closed to completely closed.	4
	Discharge (C)	
	Any amount different from normal (Does not include small amount observed in inner canthus of normal animals).	1
	Discharge with moistening of the lids and hairs just adjacent to the lids.	2
	Discharge with moistening of the lids and hairs and considerable area around eye.	3
	Score (A + B + C) x 2 Total maximum = 20	
Cornea	Opacity (A)	
	Opacity - Degree of density (area which is most dense is taken for reading).	
	Scattered or diffuse area, details of iris clearly visible.	1
	Easily discernible translucent areas, details of iris slightly obscured.	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible.	3
	Opaque, iris invisible.	4
	Area of Cornea Involved (B)	
	One quarter (or less) but not zero.	1
	Greater than one-quarter, but less than one-half.	2
	Greater than one-half, but less than three-quarters.	3
	Greater than three-quarters, up to whole area.	4
	Score equals A x B x 5 Total maximum = 80	
Iris	Values (A)	
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive).	1
	No reaction to light, hemorrhage, gross destruction (any or all of these).	2
	Score equals A x 5 Total maximum = 10	

Note: The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva.

TABLE 3

EYE IRRITATION TEST - ALBINO RABBITS

Classification of Test Materials
Based on Eye Irritation Properties

Rating	Range	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	> 0.5 - 2.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Minimally Irritating	> 2.5 - 15.0	To maintain this rating, all scores by the three day reading must be zero; otherwise, increase rating one level.
Mildly Irritating	> 15.0 - 25.0	To maintain this rating, all scores by the 7-day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	> 25.0 - 50.0	To maintain this rating, scores by 7 days must be $\leq$ 10 for 60% or more of the animals. Also, mean 7-day score must be $\leq$ 20. If 7-day mean score is $\leq$ 20 but < 60% of animals show scores < 10, then no animal among those showing scores > 10 can exceed a score of 30 if rating is to be maintained; otherwise, raise rating one level.
Severely Irritating	> 50.0 - 80.0	To maintain this rating, scores by 7 days must be $\leq$ 30 for 60% or more of the animals. Also, mean 7-day score must be $\leq$ 40. If 7-day mean score is $\leq$ 40 but < 60% of the animals show scores $\leq$ 30, then no animal among those showing scores > 30 can exceed a score of 60 if rating is to be maintained; otherwise, raise rating one level.
Extremely Irritating	> 80.0 - 110.0	

TABLE 4

EYE IRRITATION TEST - ALBINO RABBITS

with T-3421

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS 3B988	3B981	3B1012	3B1014	3B995	3B962	Means
Cornea(D-A)	1 Hour	10(1-2)	15(1-3)	5(1-1)	5(1-1)	10(1-2)	5 (1-1)	8.3
Iris		5	0	5	0	5	0	2.5
Conjunctiva (RSD)		16(2-3-3)	10(2-1-2)	14(2-2-3)	14(2-2-3)	10(2-1-2)	14(2-2-3)	13.0
	Total	31	25	24	19	25	19	23.8
Cornea(D-A)	1 Day	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20.0
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		18(3-3-3)	12(2-2-2)	16(3-2-3)	12(2-2-2)	18(3-3-3)	14(2-2-3)	15.0
	Total	43	37	41	37	43	39	40.0
Cornea(D-A)	2 Days	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	10(1-2)	18.3
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		12(2-2-2)	12(2-2-2)	14(2-2-3)	10(2-1-2)	12(2-2-2)	12(2-2-2)	12.0
	Total	37	37	39	35	37	27	35.3
Cornea(D-A)	3 Days	20(1-4)	20(1-4)	20(1-4)	20(1-4)	20(1-4)	10(1-2)	18.3
Iris		5	5	5	5	5	5	5.0
Conjunctiva (RSD)		8(2-1-1)	8(2-1-1)	12(2-2-2)	8(2-1-1)	8(2-1-1)	8(2-1-1)	8.7
	Total	33	33	37	33	33	23	32.0
Cornea(D-A)	7 Days	10(1-2)V	10(1-2)V	0	0	0	0	3.3
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		8(2-1-1)	8(2-1-1)	2(1-0-0)	0	0	0	3.0
	Total	18	18	2	0	0	0	6.3

Key: Cornea: D=Density A=Area

Conjunctiva: R=Redness S=Swelling D=Discharge

V=Vascularization

TABLE 5

EYE IRRITATION TEST - ALBINO RABBITS
with T-3421 (5 Second Contact)

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS 3B1161	3B1164	3B1148	MEANS
Cornea (D-A)	1 Hour	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		12 (2-2-2)	12 (2-2-2)	8 (2-1-1)	10.7
	Total	17	17	13	15.7
Cornea (D-A)	1 Day	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva (RSD)		8 (2-1-1)	6 (1-1-1)	6 (2-1-0)	6.7
	Total	13	11	11	11.7
Cornea (D-A)	2 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva (RSD)		0	0	0	0.0
	Total	0	0	0	0.0
Cornea (D-A)	3 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva (RSD)		0	0	0	0.0
	Total	0	0	0	0.0
Cornea (D-A)	7 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva (RSD)		0	0	0	0.0
	Total	0	0	0	0.0

Key: Cornea: D = Density
A = Area

Conjunctiva: R = Redness
S = Swelling
D = Discharge

TABLE 6

EYE IRRITATION TEST - ALBINO RABBITS
with T-3421 (30 Second Contact)

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS 3B1170	3B1162	3B1165	MEANS
Cornea (D-A)	1 Hour	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		12 (2-2-2)	16 (3-3-2)	12 (2-2-2)	13.3
(RSD)	Total	17	21	17	18.3
Cornea (D-A)	1 Day	0	0	0	0.0
Iris		5	5	5	5.0
Conjunctiva		8 (2-1-1)	10 (2-2-1)	10 (2-2-1)	9.3
(RSD)	Total	13	15	15	14.3
Cornea (D-A)	2 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)	Total	0	0	0	0.0
Cornea (D-A)	3 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)	Total	0	0	0	0.0
Cornea (D-A)	7 Days	0	0	0	0.0
Iris		0	0	0	0.0
Conjunctiva		0	0	0	0.0
(RSD)	Total	0	0	0	0.0

Key: Cornea: D = Density
A = Area

Conjunctiva: R = Redness
S = Swelling
D = Discharge

Riker Experiment No.: [illegible]

APPENDIX I

PROTOCOL

TEST: Acute Ocular Irritation Test

SPONSOR: 3M Commercial Chemical Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-3421

CONTROL ARTICLE: None

PROPOSED STARTING/COMPLETION DATE OF TEST: 6/83 - 9/83

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE:

OBJECTIVE: The objective of this test will be to determine the irritation potential of the test article to the ocular tissues (cornea, iris and conjunctiva) of 6 albino rabbits. Rabbits were selected as the test system for their sensitivity to irritants, historical use, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag which will correspond to a card affixed to the outside of the cage. The test article will be instilled into the conjunctival sac of the right eye at a dose of 0.1 with the contralateral eye of each animal serving as a control. At 1 hours and [illegible] days (additional scoring intervals may be added to further characterize the ocular reactions), the tissues will be examined and graded for irritation and injury according to a standard scoring system of Draize *et al*[b]. After completion of the test, the scores will be analyzed, and a descriptive eye irritation rating assigned to the test article. Eye examinations may be carried out with the aid of sodium fluorescein. If deemed necessary by the study director, washed eye procedures entailing a 5 and 30 second contact period with a II 1 l/min wash over a 5 min period will be conducted using 3 animals per procedure. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston-Purina, St. Louis, Missouri

[b] Draise: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics (1965) Published by the Editorial Committee of The Association of Food and Drug Officials of the United States.

RECEIVED
JUN 13 1983
SAFETY EVALUATION

W. C. McCormick 6-9-83

Sponsor Date

D. M. Marshall 6/17/83

Study Director Date

Riker Experiment No.: 0883EB0286

APPENDIX I (concluded)
Deviations and/or Amendments to Protocol

1. The source for the test animals is Hazelton-Dutchland Labs., Denver, PA.

6/21/83
Study Director Date

2.

Study Director Date

3.

Study Director Date

4.

Study Director Date

5.

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
D. M. Markoe, Jr., BS	Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

KTZ-15 (CC#34-4)
T-3421

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 25 May 83 J.H. Wilder

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes ✓ no ____

3. The stability of the test and/or control substances have been determined or will be determined as of 25 Jun 83 or end of Test J.H.W.

The above information and documentation are located in the sponsor's records.

D. Ricken 5/25/83
Sponsor Date

cc L.D. Winter 236-2B
W.C. McCormick 220-2E
W.H. Pearson 223-65E
D. Pauly 236-1

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 0883EB0286

This short term study was audited by Compliance Audit and the final report examined against the raw data on August 29, 1983. The results of the audit were reported to the study director and to management on August 30, 1983.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

D. C. Lorenz

Compliance Audit

August 30, 1983

Date

Primary Skin Irritation Test

with T-3421

in Albino Rabbits

Experiment No.: 0883EB0288

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: June 15, 1983 to June 18, 1983

Conducted By:

D. M. Markoe, Jr. 8/5/83
D. M. Markoe, Jr., BS — Date
Toxicologist
Study Director

Reviewed By:

Karen D. O'Malley 7/28/83
K. D. O'Malley, BS — Date
Senior Toxicologist
Acute Toxicology

K. L. Ebbens 8/9/83
K. L. Ebbens, BS — Date
Supervisor, Toxicology Testing

dc: M. T. Case
[redacted]
W. C. McCormick

Summary

The results of the primary skin irritation test conducted from June 15, 1983 to June 18, 1983 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3421 is moderately irritating (3.2/8.0) to the skin of female albino rabbits. Mild erythema and edema were noted at the one hour evaluation following a one day occluded contact period. The erythema persisted at the 48 hour evaluation while the edema subsided slightly.

Introduction

The objective of this study was to determine the primary skin irritation potential of T-3421 to the skin of female albino rabbits. This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits of the New Zealand breed[a] were used in the evaluation of the primary skin irritating properties of the test article. The test procedure was modeled after that of Draize et al[b].

One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart. One of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact.

The test article (0.5 g) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[c] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed[d]. One hour and 48 hours after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[c] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, Illinois.
[d] The test article was removed with acetone.

These two values were totaled and divided by four to obtain the mean primary irritation index. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with T-3421

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
3B987	2	2	2	1	2	2	2	1
3B990	2	2	2	1	2	2	1	1
3B993	2	1	1	0	2	2	2	0
3B996	2	2	1	1	2	2	2	1
3B951	2	2	2	1	2	2	2	1
3B991	2	2	2	1	2	2	2	1
Mean	2.0	1.8	1.7	0.8	2.0	2.0	1.8	0.8
Subtotal			6.3				6.6	

Rating: Moderately irritating

Primary Irritation Index: 3.2/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No.: 0883EB0288

PROTOCOL

5.

TEST: Acute Primary Skin Irritation Test

SPONSOR: **3M** Commercial Chemical **Division**

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-3421

CONTROL ARTICLE: NONE

PROPOSED STARTING/COMPLETION DATE OF TEST: 6/83 - 8/83

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: CUTTING LAB DENVER CO

OBJECTIVE: To determine the irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 grams) will be applied to 1 abraded and 1 intact site(s) on each animal, covered with gauze and secured with tape. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 48 hours after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4[b]. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

W. C. McCormick 6-9-83

Sponsor Date

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
D. M. Markoe, Jr., BS	Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
K. D. O'Malley, BS	Senior Toxicologist Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Test and/or Control Article Characterization

for

KTZ-15 (CC834-4)
T-3421

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 25 May 83

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes ✓ no ____

3. The stability of the test and/or control substances have been determined or will be determined as of 25 Jun 83 or end of Test

The above information and documentation are located in the sponsor's records.

D. Ruben 5/25/83
Sponsor Date

cc L.D. Winter 236-2B
W.C. Mc Cormick 220-2E
W. H. Pearlman 223-65E
D. Pauly 236-1

APPENDIX IV

QUALITY ASSURANCE STATEMENT

Acute Toxicology Laboratory Studies

Study No.: 082-203-227

This short term study was audited by Compliance Audit and the final report examined against the raw data on August 17, 1983. The results of the audit were reported to the study director and to management on August 17, 1983.

In addition to the data audit, different significant phases for studies underway in the Acute Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Compliance Audit on a three month schedule.

Gail E. Von Bruchhausen
Compliance Audit

August 17, 1983
Date

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-6316.17, T-6295.21, T-7132.3; ST-41
Feces Method Development Metabolism Study for Perfluorooctanesulfonate Derivatives.

Study Objective:
This study is designed to generate specimens for the 3M Environmental Laboratory Fluorine Analytical Chemistry Team to use for development and validation a method of feces metabolite analysis.

Research Client: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55144

Study Director: Andrew M. Seacat, Ph.D.
Toxicology Specialist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144
Ph.: 651-575-3161 FAX: 651-733-1773

Study Toxicologist: Deanna Luebker, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144
Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline:
In-Life Start Date: Monday November 22, 1999
In-Life End Date: Wednesday November 24, 1999

Regulatory Compliance:
This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:

Dan Hakes, Product Responsibility Liaison 3M Chemicals Division, has previously furnished high-purity N-EtFOSE, PFOS and PFOSA to the Strategic Toxicology Lab.

Identification:

Name:

- N-EtFOSE: Narrow Range N-Ethyl perfluorooctanesulfonamido ethanol, FM-3923.
- PFOS: Perfluorooctane Sulfonic Acid, Potassium Salt; CAS # 2795-39-3
- PFOSA: Perfluorooctanesulfonamide

Molecular Formula:

- N-EtFOSE: C8F17SO2N(CH2CH3)CH2CH2OH
- PFOS: $C_8F_{17}OSO_2^-K^+$
- PFOSA: $C_8F_{17}SO_2NH_2$

Lot Number:

- N-EtFOSE: Lots 30035, 30037, 30039 mixed and analyzed as one sample.
- PFOS: Lot # 217
- PFOSA: L-10009

Purity:

- **N EtFOSE:** 98 % as determined by GC, GC/MS, ^{19}F-NMR, ^{1}H-NMR and DSC techniques (1).
- **PFOS:** >99% as determined by ^{19}F-NMR (2).
- **PFOSA:**
  - Analysis by GCMS determined that the staring material was over 99% pure (3).
  - Qualitative and quantitative compositional results that were derived from the single trial ^{1}H/^{19}F-NMR cross-integration analysis revealed that the composition was 65.8% CF3(CF2)x-SO2-NH2 (Normal chain),18.7% CF3(CF2)x-CF(CF3)-(CF2)y-SO2-NH2 (Internal monomethyl branch),11.2% (CF3)2CF-(CF2)x-SO2-NH2 (Isopropyl branch), 3.5% CxF2x+1-CF(CF3)-SO2-NH2 (Alpha branch) and 0.28% $(CF_3)_3C$-$(CF_2)_x$-SO_2-NH_2 (t-Butyl branch)(4).
  - HPLC/MS characterization of the PFOSA sample revealed 9,600 ppm of PFOS, 1,100 ppm of $C_7F_{15}SO_2NH_2$, 510 ppm of $C_9F_{19}SO_2NH_2$, 6,600 ppm of $C_8F_{16}HSO_2NH_2$, 24,000 ppm of $C_{18}F_{36}HSO_2NH_2$, 1,200 ppm of $C_8F_{15}H_2SO_2NH_2$ and lower concentrations of several other amides. Based on the sum of the impurities, the purity of the PFOSA sample would be approximately 96 % (5).

Stability: Documentation will be kept on file.
Storage Conditions:
Test material will be stored tightly sealed at room temperature.
Characteristics:
Information on synthesis methods, composition or other characteristics that define the test material will be kept on file.

Animals:

Species: Rat
Strain: Sprague Dawley
Source: Harlan
Age at initiation of treatment: 9-11 weeks
Weight at initiation of treatment: approximately 200-250g
Number and sex: 12 males
GROUP 1: **control**, n=3
GROUP 2: **N-Et FOSE**, n=3
GROUP 3: **PFOS**, n=3
GROUP 4: **PFOSA**, n=3
Identification: unique tail mark

Husbandry:

Housing:
All rats will be individually housed in wire bottom metabolism cages to allow for collection of urine and feces.

Diet/Water:
Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, supplied by Harlan Teklad, Madison, WI, and tap water will be provided to all rats *ad libitum* throughout the study.

Environment:
Environmental controls for the animal room will be set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose and Dosing Procedures:

Method of administration/Dose preparation:
All rats will be dosed via oral gavage on day zero of the study using a volume of 5 ml dosing suspension / kg body weight. Rats in group 2 will receive a single 400 mg/kg dose of N-Et FOSE, rats in group 3 will receive a single 100 mg/kg dose of PFOS and rats in group 4 will receive a single 100 mg/kg dose of PFOSA. Uniform suspensions of N-Et FOSE (8%; 80 mg/mL) and PFOS (2%; 20 mg/mL) will be prepared in 2% Tween 80 using a 15-ml tissue grinder. Re-suspension of PFOS and N-EtFOSE solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing.

PFOSA (2%; 20 mg/mL) will be prepared by dissolving in acetone, then forming an emulsion in 2% Tween 80 to a final concentration of 1% acetone, 2% Tween 80. Re-suspension of PFOSA solids will be performed by pumping the emulsion through the syringe immediately before dosing. A single 5 ml / kg body weight dose of vehicle will be administered via oral gavage to rats in group 1 on day zero of the study. Two of the rats will receive 2% Tween 80 and 1 will receive a mixture of 1% acetone/2% Tween 80.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily for mortality and morbidity and notable findings will be recorded. Additional findings will be recorded as they are observed.

Body Weights:

Each animal will be weighed immediately prior to treatment and immediately prior to euthanasia.

Frequency and Number of Animals:

One control and one PFOSA rat will be sacrificed on day 1 post dose. All remaining animals will be sacrificed on day 2-post dose.

Specimen Collection & Analysis:

Method of Specimen Collection:

Urine and feces will be collected from each metabolism cage on days one and two post dose. The initial volume of urine will be recorded, the sides of the urine collection apparatus will be washed with 5-10 ml deionized water and the final volume of urine will be recorded. Daily feces weight will be recorded for each animal.

Animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, blood (≈ 6 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. Blood samples will be allowed to clot for a period of 15 to 30 minutes at room temperature, and the clot will be spun down in a centrifuge at 1100 x g for 5 minutes. The serum will be transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. Each sera sample will then be divided into 2 aliquots, transferred to a separate labeled polypropylene microfuge tube and frozen in dry ice. One aliquot will be used for metabolite analysis and the other for biochemical analysis. Each liver will be excised and weighed. The liver will be flash frozen in liquid nitrogen. A small section (approximately 3 grams) will be removed and stored at –70°C for metabolite analysis. The remainder will be stored at -70°C for biochemical analysis. Kidneys from animals sacrificed on day 2-post dose will be excised, weighed and flash frozen in liquid nitrogen for future biochemical analysis.

Specimen Handling:

All urine and feces and the serum and liver for metabolite analysis will be temporarily stored at -70°C in the Strategic Toxicology Laboratory. For analysis, these samples will be packed in dry ice and shipped to:

Kris Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
St. Paul, MN 55133-3331
Telephone No.: 651-778-6081, Facsimile No.: 651-778-6176.

All kidney specimens and the remainder of serum and liver will be kept in the Strategic Toxicology lab for biochemical analysis. These specimens will be stored at -70°C.

The number, type, and date of specimens to be generated for analysis are as follows:

TABLE 1 –Specimens for Environmental Lab

	Specimen	Collection date		Total
		Day 1 post dose	Day 2 post dose	
Environmental Lab Specimens	Urine	12	10	22
	Feces	12	10	22
	Sera	2	10	12
	Liver	2	10	12
Strategic Tox. Lab Specimens	Sera	2	10	12
	Liver	2	10	12
	Kidneys		10	10

Data Analysis:

All results will be provided for inclusion in the final report.

Responsibilities:

- Deanna Luebker and Andrew Seacat will be responsible for dosing the animals, collecting in-life specimens, performing the necropsies and collecting and sending tissue specimens for analysis.
- Andrew Seacat will draft a final report and ensure the report receives appropriate 3M review before a final report is issued.

Signatures:

Andrew M. Seacat 11/30/99

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna J. Luebker 11/24/99

Deanna J. Luebker, MS
Advanced Toxicologist
Study Toxicologist

Date

References:

1. Payfer, R. Characterization of FM-3923, Mixture of Lots 30035, 30037 & 30039 for Two Year Feeding Study. SMD Analytical Request #52489. Analytical Report # 816, 6/23/97. 3M SMD Lab Building 236-2B-11.

2. Kestner, T. Fluorochemical Isomer Distribution by ^{19}F-NMR Spectroscopy. Spectroscopy Request # 53030. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, December 1, 1997.

3. Payfer, R. GC/MS analysis of PFOSA (L-10009). SA&C Analytical Request No. 59426. Report 9/24/99. 3M SA&C Lab Building 236-2B-11.

4. Kestner, T. Chemical Characterization of PFOSA, L-10009, by 1H and 19F-NMR. Spectroscopy Request # 59426. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, September 25, 1999.

5. DeRoos F. Characterization of PFOSA Samples, T-7132-1 (L-10009) and TN-A-1584. Request # A-151254. Report 10/7/99, and Letter addendum to report 10/14/99. Corporate Analytical Technology Center, Building 201-1-29, CATC – Chromatography Group.

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-7132.2; ST-39
PHARMACOKINETIC STUDY OF PERFLUOROOCTANE SULFONAMIDE IN RATS

Study Objective:
The objective of this study is to assess the potential for oral absorption, urinary and fecal clearance and biological persistence of Perfluorooctane Sulfonamide (PFOSA) in male Sprague Dawley rats after a single oral dose. Analysis of the serum, liver, urine and feces for potential metabolites of PFOSA will be performed by LCMS and perhaps other methods. Previous studies of the N-ethyl derivative of PFOSA have concluded that PFOSA is the ultimate metabolite in rats (1,2); however, the analytical technique used in these studies was unable to detect all potential metabolites, including perfluorooctanesulfonate (PFOS). No pharmacokinetic studies have yet been done on PFOSA to resolve this issue and search for other possible metabolites in the urine and feces. Recently, validated methods have been developed for the quantitation of PFOSA and its potential metabolite, PFOS, in serum and liver down to the low part per billion level (3). One goal of this study is to determine the potential for, if occurring, the extent of conversion of PFOSA to PFOS. This information will help to explain the pharmacokinetics of NEtFOSE and its metabolites and provide data for proper risk characterization.

Research Client:
3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Sponsor:
3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Study Location:
3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55144

Study Director:
Andrew M. Seacat, Ph.D.
Toxicology Specialist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144
Ph.: 651-575-3161 FAX: 651-733-1773

Study Toxicologist:
Deanna Luebker, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144

Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline

In-Life Start Date: October 4th, 1999
In-Life End Date: November 2nd, 1999
Analytical Completion Date: TBA
Final Report Completion Date: TBA

Regulatory Compliance:

This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:

Dan Hakes, Product Responsibility Liaison 3M Chemicals Division, will furnish high-purity PFOSA.

Identification:

Name: Perfluorooctanesulfonamide
Molecular Formula: $C_8F_{17}SO_2NH_2$
Lot Number: L-10009 (Prepared by George Moore, 3M SMD Lab, Bldg 236, April 1996)
Purity: Analysis by GCMS determined that the staring material was over 99% pure (4). Qualitative and quantitative compositional results that were derived from the single trial $^1H/^{19}F$-NMR cross-integration analysis revealed that the composition was 65.8% CF3(CF2)x-SO2-NH2 (Normal chain),18.7% CF3(CF2)x-CF(CF3)-(CF2)y-SO2-NH2 (Internal monomethyl branch),11.2% (CF3)2CF-(CF2)x-SO2-NH2 (Isopropyl branch), 3.5% CxF2x+1-CF(CF3)-SO2-NH2 (Alpha branch) and 0.28% $(CF_3)_3C$-$(CF_2)_x$-SO_2-NH_2 (t-Butyl branch)(5). HPLC/MS characterization of the PFOSA sample revealed 9,600 ppm of PFOS, 1,100 ppm of $C_7F_{15}SO_2NH_2$, 510 ppm of $C_9F_{19}SO_2NH_2$, 6,600 ppm of $C_8F_{16}HSO_2NH_2$, 24,000 ppm of $C_{18}F_{36}HSO_2NH_2$, 1,200 ppm of $C_8F_{15}H_2SO_2NH_2$ and lower concentrations of several other amides. Based on the sum of the impurities, the purity of the PFOSA sample would be approximately 96 % (6 & appendix I).

Stability:

Documentation will be kept on file with the Sponsor.

Storage Conditions:

Upon receipt, test material will be stored tightly sealed at room temperature.

Characteristics:

Information on synthesis methods, composition or other characteristics that define the test material will be kept on file with the Sponsor.

Animals:

Species: Rat
Strain: Sprague Dawley
Source: Harlan
Age at initiation of treatment: 6-8 weeks
Weight at initiation of treatment: approximately 150-250g
Number and sex: 30 males

Table 1 - Dose Groups

Group	Dose	N	Euthanasia
1	0 mg/kg	15	5 each on days 1, 4 and 29 post dose
2	5 mg/kg	15	5 each on days 1, 4 and 29 post dose

Identification: ear tag with animal number or unique tail mark.
AUA Number: 2246

Husbandry:

Housing:

All rats from groups 1 and 2, which are to be sacrificed on day 29 post dose, will be housed individually in metabolism cages for portions of the study (see Table 2). When not in metabolism cages, these rats will be group housed in standard cages. All other rats will be group housed in standard cages throughout the study.

Diet/Water:

Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, supplied by Harlan Teklad, Madison, WI, and tap water will be provided to all rats *ad libitum* throughout the study.

Environment:

Environmental controls for the animal room will be set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose and Dosing Procedures:

Method of administration/Dose preparation:

A single 5mg/kg dose of PFOSA will be administered via oral gavage to rats in group 2 on day zero of the study. The PFOSA will be prepared from a stock solution of 100mg/ml PFOSA in acetone. A final 0.1% (1mg/ml) uniform suspension (or emulsion) of PFOSA in 2% Tween 80 and 1% acetone will be prepared using a 15 ml dounce tissue grinder. A volume of 5 ml suspension / kg body weight will be administered to each rat. Re-suspension of solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing. A single 5

ml / kg body weight dose of vehicle, 2% Tween 80 and 1% acetone, will be administered via oral gavage to rats in group 1 on day zero of the study.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily (excluding weekends and holidays) for mortality and morbidity and notable findings will be recorded. Additional findings will be recorded as they are observed.

Body Weights:

Each animal will be weighed immediately prior to treatment, weekly thereafter and immediately prior to euthanasia.

Specimen Collection:

Frequency (See table 2):

Urine and feces collections will be made on days 1 - 4 post dose.
Necropsies will be performed on days 1, 4 and 29 post dose.

Table 2 - Schedule

Sun	Mon	Tues	Wed	Thurs	Fri	Sat
Oct 3	Oct 4 day 0 DOSING	Oct 5 day 1 PD Collection, Dy 1 PD sac	Oct 6 day 2 PD Collection	Oct 7 day 3 PD Collection	Oct 8 day 4 PD Collection Switch to reg cages. Dy 4 PD sac.	Oct 9 day 5 PD
Oct 10 day 6 PD	Oct 11 day 7 PD	Oct 12 day 8 PD	Oct 13 day 9 PD	Oct 14 day 10 PD	Oct 15 day 11 PD	Oct 16 day 12 PD
Oct 17 day 13 PD	Oct 18 day 14 PD	Oct 19 day 15 PD	Oct 20 day 16 PD	Oct 21 day 17 PD	Oct 22 day 18 PD	Oct 23 day 19 PD
Oct 24 day 20 PD	Oct 25 day 21 PD	Oct 26 day 22 PD	Oct 27 day 23 PD	Oct 28 day 24 PD	Oct 29 day 25 PD	Oct 30 day 26 PD
Oct 31 day 27 PD	Nov 1 day 28 PD	Nov 2 day 29 PD Dy 29 PD sac.				

Method of Specimen Collection:

Urine and feces will be collected from each metabolism cage at the designated times. The initial volume of urine will be recorded, the sides of the urine collection apparatus will be washed with approximately 5-10ml deionized water and the final volume of urine will be brought to 15 ml with additional deionized water. Daily feces weight will be recorded for each animal. At the designated times, animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, blood ($\approx$ 6 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. Blood samples will be allowed to clot for a period

of 15 to 30 minutes at room temperature and the clot will be spun down in a centrifuge at 1100 x g for 5 minutes. The serum will be transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. Each sera sample will then be transferred to a separate labeled polypropylene microfuge tube and frozen in dry ice. Livers will be removed, weighed, placed individually into labeled sterile sample bags and flash frozen in liquid nitrogen then maintained on dry ice.

Specimen Handling:

Specimens will temporarily be stored in a freezer set to maintain -60 to -80°C. For metabolite analysis, these specimens will be packed in dry ice and shipped to:

Kris Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
St. Paul, MN 55133-3331
Ph: 612-778-6081, FAX: 612-778-6176.

All results will be provided for inclusion in the final report.

The number, type and date of collection of specimens to be generated for analysis are as follows:

Table 3 - Specimens

Specimen	day 1 post dose	day 2 post dose	Day 3 post dose	day 4 post dose	day 29 post dose	Total
Serum (5/group/day)	10			10	10	30
Liver (5/group/day)	10			10	10	30
Urine (5/group/day)	10	10	10	10		40
Feces (5/group/day)	10	10	10	10		40

Data Analysis:

Data collected on parent compound and identifiable metabolites will be analyzed for toxicokinetic parameters and for statistically significant differences between groups using ANOVA and /or Students T-test.

Responsibilities:

- Deanna Luebker and Andrew Seacat will be responsible for dosing the animals, collecting in-life specimens, performing the necropsies and collecting and sending tissue specimens for analysis.
- Kris Hansen, 3M Environmental, will be responsible for analytical evaluation of the
- Andrew Seacat will draft a final report and ensure the report receives appropriate 3M review before a final report is issued.

Signatures:

Andrew M. Seacat 10/27/99

Andrew M. Seacat Ph.D.
Toxicology Specialist
Study Director

Date

Deanna J Luebker 10/27/99

Deanna Luebker, MS
Advanced Research Toxicologist
Study Toxicologist

Date

Daniel C Hall 11/1/99

Sponsor Representative

Date

References:

1. Grossman M.R. and Bowen J.M. (1990) Tissue analysis of fluorinated sulfonamide pesticide: an evaluation of distribution, elimination, and potential for bioaccumulation in orally exposed rats. M.S. Thesis, Univ. of Georgia, Athens, GA. (also possibly published as: Grossman Mark R. and Bowen J.M. (1990) Tissue distribution and elimination of a fluorinated sulfonamide pesticide in rats. *Fundam. Appl. Toxicol., but not found).*

2. Grossman M.R., Mispagel, M.E. and Bowen J.M. (1992) Distribution and tissue in rats during and after prolonged dietary exposure to a highly fluorinated sulfonamide pesticide. *J. Agric. Food Chem.* 40, 2505 – 2509.

3. K.J. Hansen, L.A. Clemen, M.E. Ellefson, H.O. Johnson. (1999). Compound Specific Characterization of Organic Fluorochemicals in General Population Human Sera Samples. 3M Environmental Lab, St. Paul, MN 55133.

4. Payfer R.M. GC/MS analyses of PFOSA (L-10009). SA&C Analytical Request No. 59426. Report 9/24/99. 3M SA&C Lab Building 236-2B-11.

5. Tom Kestner Chemical Characterization of PFOSA, L-10009, by 1H and 19F-NMR Spectroscopy Requests # 59426. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, September 25, 1999.

6. DeRoos F.L. Characterization of PFOSA Samples, T-7132-1 (L-10009) and TN-A-1584. Request # A-151254. Report 10/7/99. Corporate Analytical Technology Center, Building 201-1-29, CATC – Chromatography Group.

Appendix I:

Tel: 736-0665
201-1W-29

Corporate Analytical Technology Center

To: Larry A. Wendling/US-Corporate/3M/US
cc: Andrew Seacat/US-Corporate/3M/US
Subject: Characterization of PFOSA Sample for Toxicology

Larry,

I have completed the HPLC/MS characterization of the PFOSA sample (T-7132-, L-10009 prepared by G. Moore 4/96) that is proposed to be used for the animial feeding study. **I found 9,600 ppm of PFOS, 1,100 ppm of $C_7F_{15}SO_2NH_2$, 510 ppm of $C_9F_{19}SO_2NH_2$, 6,600 ppm of $C_8F_{16}HSO_2NH_2$, 24,000 ppm of $C_{18}F_{36}HSO_2NH_2$, 1,200 ppm of $C_8F_{15}H_2SO_2NH_2$ and lower concentrations of several other amides. Based on the sum of the impurities, the purity of the PFOSA sample would be approximately 96 %.**

I've thought a little more about apparent presence of $C_{18}F_{36}HSO_2NH_2$ in the sample. The identification of this compound was based primarily on the observed molecular weight and the relative elution order in the chromatogram. Rather than $C_{18}F_{36}HSO_2NH_2$, it seems more probable that this compound is actually $(C_8F_{17}SO_2)_2NH$. Confirmation of this identification will require additional analyses. I also need to think more about whether the other partially hydrogenated perfluoroamides may actually be of this

The PFOS was quantified using a standard curve prepared by analyzing PFOS standards so its concentration should be accurate. The amide impurities, however, including the partially hydrogenated amides, were all quantified using a PFOSA standard curve assuming that they had the same mass spectral response factor as did PFOSA. While this assumption will introduce some error to the quantitative data, it is the best we can do since we do not have standards for each of these amides. Also, this calculated purity assumes that all of the impurities are amenable to HPLC and are detected by the analysis. While not always true, this sample was analyzed by Rick Payfer using GC/MS and by CATC using our GC method. Neither of the analyses found volatile or semivolatile impurities, e.g., N-methyl FOSE, N-ethyl FOSE, etc. at concentrations greater than 50 ppm.

I also carried out a semiquantitative assessment of the purity of the PFOSA by comparing the PFOSA response to a PFOSA standard curve. Using this technique, the purity of the PFOSA sample was found to be 118 %. These analyses were carried out in duplicate, with triplicate injections of each solution, so they should be relatively accurate as analytical variation would be averaged. In general, however, it is not highly accurate to use a chromatographic method to quantify a relatively pure material due to the extremely large dilution factor, in this case >100,000, that must be applied. It is not expected that instrumental variation would bias the purity high. It is possible that the purity of the sample is actually higher than the purity of the PFOSA that we are using as a standard! If this were true, the purity would be determined to be > 100%.

Andrew Seacat has reviewed the concentrations of the impurities that were determined and calculated that the concentration of PFOS will not adversely affect the study. At 9,600 ppm, he calculates that the rats receiving 3 mg/kg/day would ingest app. 0.20 mg PFOS total in

Appendix I Continued:

28 days assuming a 0.25 kg rat. If we further assume that 30 % of that is deposited in the liver (based on previous feeding studies) and liver is approximately 12 grams, then the predicted concentration in the liver from the residual PFOS would be 200 ug PFOS x 0.3 / 12 g liver weight = 5 ug/gram, or 5 ppm, which in itself should not impose any toxicological consequences. Any PFOS measured significantly over that could be attributed to metabolism of PFOSA to PFOS.

He believes that the 1-H and 2-H perfluoro amides are interesting as they may be subject to metabolic attack, however these amides should be analytically distinct from the Perfluorinated species on down the line, and would not amount to a foreseeable toxic concentration and therefore would be acceptable if no other substitute can be prepared. Andrew is still thinking about whether the $(C_8F_{17}SO_2)_2NH$ will have a negative impact on the study.

Please give me a call at 6-0665 if you have any questions concerning our analyses or if I can provide additional information.

Fred L. DeRoos
CATC

Pathology Associates International
A Company of Science Applications International Corporation
An Employee-Owned Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL

Study Title:

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluoroctanesulfonamide (PFOSA 3M T-7091.1) in Rats

Date:

January 12, 1999

Performing Laboratory

R.O.W. Sciences
15 Firstfield Road
Gaithersburg, Maryland 20878

Laboratory Study Identification:

Study Number: (1132-100)

PAI Project Number: (Histology number to be assigned by PAI by protocol amendment)

15 Worman's Mill Court, Suite I * Frederick, Maryland 21701 * (301) 663-1644 * (301) 663-8994 FAX

Study

Cell Proliferation Study with N-Ethyl Perfluorooctanesulfonamido Ethanol (N-EtFOSE; 3M T-6316.11), Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; 3M T-6295.16), and N-Ethyl Perfluoroctanesulfonamide (PFOSA 3M T-7091.1) in Rats

Purpose

To assess cell proliferation and peroxisome proliferation in rats administered test material in the diet.

Sponsor

3M Corporate Toxicology
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

Study Representative

Marvin T. Case, D.V.M, Ph.D.
3M Corporate Toxicology
Phone No.: 651 733-5180
Fax No.: 651 733-1773
Email: mtcase@mmm.com

Alternative Study Representative

Andrew M. Seacat, Ph.D.
3M Corporate Toxicology
Phone No.: 651 575-3161
Fax No.: 651 733-1773
Email: amseacat@mmm.com

Study Location

R.O.W. Sciences
15 Firstfield Road
Gaithersburg, Maryland 20878

Study Monitor

Sandra R. Eldridge, Ph.D.
Pathology Associates International
Phone No. 301 624-2036
Fax No. 301 663-8994
Email: srepaisaic@aol.com

Study Director

Gary W. Wolfe, Ph.D., D.A.B.T.
R.O.W. Sciences
Phone No.: 301 330-3723
Fax. No.: 301 330-3738
Email: gwolfe@lab.row.com

Principal Investigator

Sandra R. Eldridge, Ph.D.
Pathology Associates International
Phone No. 301 624-2036
Fax No. 301 663-8994
Email: SREPAISAIC@aol.com

Study Pathologist

Carolyn Moyer, D.V.M., Diplomate, A.C.V.P.
Pathology Associates International
Phone No. 301 624-2928
Fax No. 301 663-8994

Proposed Study Timetable

In-life Start Date: To be added by protocol amendment; Day 0
In life End Date: To be added by protocol amendment
Audited Draft Report Date: To be added by protocol amendment

Regulatory Compliance

This study will be conducted in the spirit of Good Laboratory Practice (GLP) regulations.

Animal Care and Use Statement

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

Quality Assurance

Not applicable.

Test Materials

Test Material:	N-EtFOSE (completed by 3M)	PFOS (completed by 3M)	PFOSA (to be added by protocol amendment)	Wy-14,643 (to be added by protocol amendment)
Identification:	N-EtFOSE	PFOS	PFOSA	Wy
Lot Number:	FM 3929	217		
Purity:	99.2%	99%		
Stability:	> 5 years	> 5 years	> 5 years	
Storage Conditions:	room temp.	room temp.	room temp.	
Characteristics:	waxy solid	white powder	amber waxy solid	

Reserve (Archive) Samples

A reserve sample (approximately 5 g) of each lot will be taken and stored at room temperature. These samples will be transferred to the Sponsor after completion of the in-life phase to be retained in accordance with 40 CFR 792.195.

Disposition of Test Material

After authorization from the Sponsor, any remaining test material will be returned to:

Marvin Case, D.V.M., Ph.D.
3M Corporate Toxicology
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000
Phone No.: 651-733-5180
Fax No.: 651-733-1773

Animals

Species:	Rat
Strain:	Crl:CD®(SD) IGS BR
Source:	Charles River Laboratories, Inc., Raleigh, NC
Age at Initiation of Treatment:	Preferable 6 weeks of age, but not more than 8 weeks of age
Weight at Initiation of Treatment:	150 to 300 g
Number and Gender:	males
Identification:	unique identification by individual car tags and cage cards

Husbandry

Housing:	Single housed in hanging stainless steel wire cages
Diet:	Teklad 7012 Certified Rodent Diet. Fresh food will be provided weekly. Feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinate hydrocarbons, organophosphates, and specified nutrients. Specified nutrients analyses are on file at R.O.W. Sciences.
Water:	Tap water, provided ad libitum via an automatic watering system or water bottles. The water is analyzed at least two times per year for contaminants and specific microbes. The results of these analyses are on file at R.O.W. Sciences.
Contaminants:	The study director and/or the Sponsor have considered possible interfering substances potentially present in animal feed and water, including the test material itself or possible structurally related materials as well as the items listed in (2) and (3) above. None of these contaminants are reasonably expected to be present in animal feed or water at levels sufficient to interfere with this study.
Environment:	The targeted temperatures are between 64 and 79°F with a relative humidity between 30% and 70%. Temperature and humidity are monitored continuously. A 12-hour light/12-hour dark cycle will be maintained. Ten or greater air changes/hour will be maintained.
Acclimation:	Animals will be acclimated to the facility for a minimum of 7 days prior to the start of dosing. Animals will be observed for general health and suitability for testing during this period. Animals that are diseased or unsuitable for testing will be removed from the study.
Randomization:	Using computer-generated random numbers wit assignment to groups, At the time of randomization, the weight variation of the animals of each sex used should not exceed ± 2 S.D. of the mean weight, and the mean body weights for each group of each sex will not be statistically different.
Justification:	Rats will be used because of the extensive historical data base, and the FDA requirements for a rodent species.

Group Designations, Dietary Levels and Scheduled Sacrifice Time Points

Group Number (Time Point)	Number of Male Rats							
	Control (0 ppm)	N-EtFOSE 300 ppm	N-EtFOSE 100 ppm	N-EtFOSE 30 ppm	PFOS 20 ppm	PFOSA 100 ppm	Wy-14,643 1000 ppm	Total No. of Animals
1 (48 hrs)	10	10	10	10	10	10	5	65
2 (7 days)	10	5	5	5	5	5	5	40
3 (14 days)	10	5	5	5	5	5	5	40
4 (1 wk recovery)	10	5	5	5	5	5	5	40
5 (4 wk recovery)	10	5	5	5	5	5	5	40
Total No. of Animals	50	30	30	30	30	30	25	225

Dosing Procedures

Method of Administration

Dietary. Animals in Groups 1 through 3 will receive test diet for 48 hours, 7 days, and 14 days, respectively.

Animals in Groups 4 and 5 will receive test diet for 14 days followed by a 1 or 4 week recovery period, respectively.

Reason for Dosing Route

The potential human exposure is by the oral route.

Dose Preparation

Before initiation of treatment, dose preparation of each test material will be mixed. All dose preparations will be stored at room temperature. Dose preparation will be documented and reported. See Attachment I for test diet preparation procedures.

Retention Sample

Samples (approximately 100 g) will be taken from the dose preparation and stored at room temperature. Unless used for analyses, these samples will be discarded at least 1 month after completion of the in-life phase.

Observation of Animals

Clinical Observations

Each animal will be observed twice daily (a.m. and p.m.) for mortality and moribundity; findings will be recorded as they are observed.

Body Weights

Prior to treatment (at randomization), weekly for Week 1 through 4 weeks of recovery.

Food Consumption

Weekly for Week 1 through 4 weeks of recovery.

Clinical Chemistry

Animals will be fasted overnight before animal's scheduled necropsy; blood will be collected from a jugular vein into an EDTA-coated tube. Serum enzyme levels of alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), cholesterol and triglycerides will be determined.

Termination

Unscheduled Sacrifices and Deaths

Necropsies will be done. Animals to be sacrificed will be anesthetized with CO_2, weighed, and exsanguinated.

Scheduled Sacrifices

Interim Sacrifices

At 48 hrs, 7 days, and 14 days, animals will be fasted overnight, bled for serum samples, anesthetized with CO_2, weighed, and exsanguinated.

NOTE: Two serum samples will be needed, (1) a 0.5 ml sample for clinical chemistry and (2) a 1.5 ml sample for compound level analysis.

The abdominal cavity of each animal will be opened, the liver will be removed and weighed, and liver samples will be collected. Animals will be discarded after liver collection.

Terminal Sacrifices

After 1 and 4 weeks of recovery, animals will be fasted overnight, bled for serum samples, anesthetized with CO_2, weighed, exsanguinated, and necropsied.

NOTE: Two serum samples will be needed, (1) a 0.5 ml sample for clinical chemistry and (2) a 1.5 ml sample for compound level analysis.

Postmortem Procedures

Necropsy

The necropsy will include an examination of the external features of the carcass; all external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Cell Proliferation Tissue Collection and Immunohistochemical Evaluation

Representative samples of the left lateral lobe of the liver and any macroscopic lesions of the liver will be collected and preserved in zinc formalin.

After fixation, each sample of liver will be delivered to:

Sandra R. Eldridge, Ph.D.
Pathology Associates International
15 Worman's Mill Court, Suite I
Frederick, Maryland 21701

Proliferation cell nuclear antigen (PCNA) evaluation will be done on the samples. In addition, liver sections prepared from the same tissue block will be stained with hematoxylin and eosin and examined microscopically.

Palmitoyl-CoA Oxidase Tissue Collection and Analyses

A sample (approximately 500 mg) of the right lateral lobe of the liver will also be collected from select animals and flash-frozen in liquid nitrogen. See Attachment II for procedure. The liver tissue will be stored in a freezer set to maintain -60 to -80° C until analyzed by Covance for palmitoyl-CoA Oxidase activity. The liver samples to be analyzed will include all study animals, EXCEPT for the Wy-14,643 animals and all animals from the 4-week recovery groups. In addition to this study, samples from a previous 3M study will be analyzed for palmitoyl-CoA Oxidase activity; these samples consist of liver samples from 35 rats and 35 guinea pigs.

Tissue Collection for Electron Microscopic Evaluation

Sections of liver from all animals will be collected, minced to approximately one millimeter cubes and placed in a fixative appropriate for electron microscopy. The containers and fixative will be provided by PAI. Electron microscopy will be performed on one animal per treatment group exhibiting the highest cell proliferative response as well as one control animal at the discretion of the Sponsor, from one time point as well as the 4-week recovery. Thus, EM will be performed on one animal from the control, N-EtFOSE (one dose only to be determined), PFOS, PFOSA, and Wy groups at one of the time points, as well as the 4 week recovery, for a total of 10 animals.

Remaining Liver Tissue

The remaining liver tissue will be frozen and stored at -60 to -80° C for possible future analysis.

Organ Weights

At the scheduled sacrifices, the liver will be weighed.

Histopathology

Liver from each animal that is examined for cell proliferation will be stained with hematoxylin and eosin, and examined microscopically for histopathologic changes.

Reports

One copy of the draft report will be sent to the Sponsor. The report will include the following information:

Experimental Design and Methods

Results

dose analyses
mortality
clinical observations
body weights
body weight changes
food consumption
test material consumption
clinical pathology results
palmitoyl-CoA oxidase activities
macroscopic observations
microscopic observations
ultrastructural observations
cell proliferation assessments

Record Retention

All raw data, documentation, records, protocol, specimens, and final report generated as a result of this study will be archived in the storage facilities of PAI for a period of 1 year following submission of the final report to the Sponsor. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor and a return fee will be charged. All raw data stored on magnetic media will be retain by PAI.

PROTOCOL APPROVAL

Marvin T Case — 13 Jan 1999

Marvin Case, D.V.M., Ph.D. — Date
Study Representative
3M Corporate Toxicology

Gary W. Wolfe — 1/12/99

Gary W. Wolfe, Ph.D., D.A.B.T — Date
Study Director
R.O.W. Sciences

1-12-99

Sandra R. Eldridge, Ph.D. — Date
Principle Investigator
Pathology Associates International

Attachment: I
Test Diet Preparation Procedures

1) Determine the amount of test diet (feed) that is to be prepared and weigh out that amount of feed.
2) Calculate the amount of test article that is needed to prepare the test diet at the desired concentration.
3) Accurately weigh out the necessary amount of test article.
4) Transfer the weighed test article to a container and add a small volume of acetone to container. Manually mix to dissolve the test material adding acetone as necessary (typical ratio of test material to acetone is 1 g: 15-20 ml acetone). Visually inspect test material/acetone for solubility of test material.
5) Prepare a pre-mix by transferring the dissolved test material into 4 kg of feed in a Hobart mixing bowl. Mix for 10 minutes. Transfer the premix to a larger mixer, add remaining amount of weighed diet, mix for 30 minutes.

Attachment: II
Collection of Tissue Samples for Biochemical and Molecular Analysis

Because of the extreme instability of certain enzymes and biomolecules, it is essential that tissues be harvested as soon after death as possible and flash frozen immediately in liquid nitrogen. Failure to follow these procedures may lead to loss of the entire sample and all the energies and resources that were invested into generating the samples. Therefore, make every effort to comply with the following:

1) Harvest the tissue samples as soon as possible after death. Delays may allow for biodegradation and/or inactivation of the desired endpoint.
2) Immediately submerse the tissue sample directly into liquid nitrogen. Dry ice or other alternatives will not suffice. It is important that the tissue be immersed directly in liquid nitrogen Transferring it to a dry vessel (or sample container) suspended in liquid nitrogen will not suffice. The tissue may freeze to the vessel wall and will then be impossible to remove without completely destroying the vessel (or sample container).
3) Be absolutely sure to maintain the tissue frozen. It should be stored in a sealed container at -70°C and shipped or transferred on dry ice. If needed, the frozen sample can be fractured (broken into portions for different applications) by placing in a crucible which contains liquid nitrogen to keep the sample frozen while grinding/fracturing.

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

3. PFOSAA	Perfluorooctane sulfonylamido (ethyl)acetate

Acute Toxicity

1) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 77-1108A, 3M Reference No. T-1983 (FC-128, potassium salt 100%, solid), January 5, 1978

2) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

3) Primary Eye Irritation Study – Rabbits, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

4) Primary Skin Irritation Study – Rabbits, Biosearch, Inc., Project No. 77-1127A, 3M Reference No. T-2001 (FC-128), January 6, 1978

5) An Acute Inhalation Toxicity Study of T-2307 CoC in the Rat, Bio/dynamics, Inc., Project No. 78-7186, 3M Reference No. T-2307 (FC-128), February 8, 1979

Additional Acute Toxicity Studies Not Submitted (Bibliography only)

1) Acute Oral Toxicity – Rats, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129, approximately 40-50% in solution), March 2, 1978

2) Primary Eye Irritation Study – Rabbits, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129), March 2, 1978

3) Primary Skin Irritation Study – Rabbits, Biosearch, Inc., Project No. 78-1191A, 3M Reference No. T-2081 (FC-129), March 2, 1978

4) Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088AR0362, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November 5, 1982

5) Primary Skin Irritation Test with T-3290CoC in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088EB0423, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), October 15, 1982

6) Acute Ocular Irritation Test with T-3290CoC in Albino Rabbits, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 088EB0424, 3M Reference No. T-

3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), October 26, 1982

Genotoxicity

1) Bacterial Reverse Mutation Test of ν-1, Hita Research laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute, Study Code: K01-1815, Report No. T-4663, 3M Reference No. T-6668.1, FC-129 (approximately 40-50% in solution of water and organic solvent), October, 1996

2) In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3290CoC, SRI International, Project No. 3145, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November, 1982

Pharmacokinetic Studies

1) 28 Dermal Percutaneous Absorption Study with FC-128 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0979AB0629, 3M Reference No. T-3991, March 15, 1981

2) 28 Day Dermal Percutaneous Absorption Study with FC-129 in Albino Rabbits, Safety Evaluation laboratory, Riker Laboratories, Inc., Project No. 0979AB0627, 3M Reference No. T-3989, March 14, 1981

3) Final Report – Analytical Study: Single-Dose Dermal Absorption / Toxicity Study of T-6051 and T-6054 in Rabbits, 3M Environmental Laboratory, Study No. ADMT-013195.1, in vivo Study Reference No. HWI 6329-133 (Hazleton Wisconsin, Inc.), 3M Reference Nos. T-6051 (FC-129 treated fabric) and T-6054 (FC-129 solution), November 22, 1995

4) Final Report, Analytical Report and Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits, 3M Environmental Technology & Services, In-Vivo Study Reference No. HWI#6329-138, Study No. AMDT-122094.2, 3M Reference No. FC-129, November 22, 1995

Studies in Progress

1) Corporate Toxicology Study Outline, FC-129 Preliminary ADME Screen in Rats, 3M Strategic Toxicology Laboratory, July, 1998

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

Pre-1976 Studies (bibliography only)

1) Skin and Eye Irritation Assay Report, WARF Institute, Project No. 2031046, 3M Reference No. FC-128, April 24, 1962 (plus December 29, 1966 letter containing individual eye scores)

FC-128

BIOSEARCH, INC. p.o. box 8598 philadelphia, pennsylvania 19101
telephone: (215) 848-4499
Project Number - 77-1108A

Submitted to: 3M Company
3M Center
St. Paul, Minnesota
55101

Material: 3M Company - T-1983CoC

Sample Received: 11/2/77 Study Initiated: 11/18/77 Study Completed: 12/15/77

Date of Report: 1/5/78

Test: Acute Oral Toxicity - Rats

Object of Test: To study the acute oral toxicity in rats of the subject material.

Procedure: Four groups of 5 male & 5 female albino rats of the Sherman-Wistar Strain weighing between 200 and 300 gm were employed in this study. The rats were deprived of food but not water for 24 hours prior to dosing. Each animal was weighed and dosed by direct administration of the experimental material into the stomach by means of a syringe and dosing needle.

The sample was dosed as a 50% w/v suspension in water.

The following dosage levels were administered:

1.25 ml/kg.
2.50 ml/kg.
5.0 ml/kg.
10.0 ml/kg.

Following administration the animals were allowed food and water ad libitum for the 14 day observation period during which time the rats were observed for signs of toxicity and mortalities.

Results: See Table 1.

Conclusion: The subject material when studied in male and female albino rats has an acute oral LD50 of approximately 2.5 ml/kg of a 50% w/v suspension in water or 1.25 gm/kg. of the original sample.

Karl L. Gabriel

000180

Karl L. Gabriel, V.M.D., Ph.D.
Director

TABLE 1

Acute Oral Toxicity

Material: 3M Company - T-1983CoC, as a 50% w/v suspension in water.

Dosage Level ml/kg	Number of Animals Dosed	Mortalities Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Dead 14 Days	Total Survived 14 Days	Initial Weight gm	Final Weight gm
1.25	5 males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	230	260
1.25	5 females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	250	275
2.50	5 males	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2	3	250	215
2.50	5 females	0	2	0	0	0	0	0	0	1	0	0	0	0	0	3	2	240	220
5.0	5 males	5	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	235	-
5.0	5 females	4	1	-	-	-	-	-	-	-	-	-	-	-	-	5	0	250	-
10.0	5 males	5	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	240	-
10.0	5 females	5	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	265	-

The LD_{50} is approximately 2.5 ml/kg. of a 50% w/v suspension in water or 1.25 gm/kg. of the original sample.

At 1.25 ml/kg. (0.625 gm/kg.) the animals were slightly lethargic for 24 hours and then appeared normal. At 2.5 ml/kg. (1.25 gm/kg.) the animals were depressed after 2 hours and did not regain normalcy throughout the observation period. Some deaths occurred as noted and the survivors were dehydrated and had lost weight. At 5.0 ml/kg. and 10.0 ml/kg. (2.5 gm/kg. & 5.0 gm/kg.) the animals were severely depressed after 30 minutes and comatose within 2 hours.
Gross pathologic examination revealed nothing remarkable.

FC-128

BIOSEARCH, INC. p.o. box 8598 philadelphia, pennsylvania 19101
telephone: (215) 848-4499
Project Number - 77-1127A

Submitted to:

3M Company
3M Center
St. Paul, Minnesota
55101

Material: 3M Company - T-2001CoC

Sample Received: 11/21/77 Study Initiated: 11/23/77 Study Completed: 12/29/77

Date of Report: 1/6/78

Test: Acute Oral Toxicity - Rats

Object of Test: To study the acute oral toxicity in rats of the subject material.

Procedure: Four groups of 5 male & 5 female albino rats of the Sherman-Wistar Strain weighing between 200 and 300 gm were employed in this study. The rats were deprived of food but not water for 24 hours prior to dosing. Each animal was weighed and dosed by direct administration of the experimental material into the stomach by means of a syringe and dosing needle.

The sample was dosed as supplied.

The following dosage levels were administered:

0.50 ml/kg.
2.50 ml/kg.
3.75 ml/kg.
5.00 ml/kg.

Following administration the animals were allowed food and water ad libitum for the 14 day observation period during which time the rats were observed for signs of toxicity and mortalities.

Results: See Table 1.

Conclusion: The subject material when studied in male and female albino rats has an acute oral LD50 between 0.5 ml/kg. and 2.5 ml/kg.

Karl L. Gabriel

000182

Karl L. Gabriel, V.M.D., Ph.D.
Director

TABLE I

Acute Oral Toxicity

Material: 3M Company - T-2001CoC, as supplied.

Dosage Level ml/kg	Number of Animals Dosed	Mortalities Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total Dead 14 Days	Total Survived 14 Days	Initial Weight gm	Final Weight gm
0.50	5 males	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	215	245
0.50	5 females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	230	255
2.50	5 males	3	1	0	1	-	-	-	-	-	-	-	-	-	-	5	0	225	-
2.50	5 females	2	3	-	-	-	-	-	-	-	-	-	-	-	-	5	0	250	-
3.75	5 males	3	1	0	0	0	1	-	-	-	-	-	-	-	-	5	0	210	-
3.75	5 females	4	1	-	-	-	-	-	-	-	-	-	-	-	-	5	0	235	-
5.00	5 males	5	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	220	-
5.00	5 females	4	1	-	-	-	-	-	-	-	-	-	-	-	-	5	0	240	-

The LD_{50} is between 0.5 ml/kg. and 2.5 ml/kg.

At 0.50 ml/kg. the animals were slightly depressed for 4-6 hours after dosing. At the three higher dosage levels the animals were comatose within 30 minutes to 4 hours and died as noted.
Gross pathologic examination revealed nothing remarkable.

FC-128

BIOSEARCH, INC. p.o. box 8598 philadelphia, pennsylvania 19101
telephone: (215) 848-4499

Project Number - 77-1127A

Submitted to:

3M Company
3M Center
St. Paul, Minnesota
55101

Material: 3M Company - T-2001CoC

Sample Received: 11/21/77 Study Initiated: 11/30/77 Study Completed: 12/6/77

Date of Report: 1/6/78

Test: Primary Eye Irritation Study - Rabbits

Object of Test: To determine the degree of irritation, if any, which the subject material may produce when instilled into the eyes of albino rabbits.

Method of Test: The methods employed in the testing, evaluation and in the grading of the test material are those described in Section 1500.42 - Hazardous Substances and Articles, Administration and Enforcement Regulations, Federal Register, Vol. 38. No. 187, P. 27019, 27 September 1973.

The sample was used as supplied.

Six healthy young adult albino rabbits were used in this study. 0.1 ml of the experimental material was instilled into the right eyes of the test animals while the other eyes remained untreated to serve as controls. The test material was not washed from the eyes.

The treated eyes were examined at 1, 24, 48 & 72 hrs. & 5 & 7 days following instillation of the test material into the eyes. Interpretation of the results was made in accordance with the grading system outlined in the "Illustrated Guide for Grading Eye Irritation By Hazardous Substances".

Results: See Table 1.

Conclusion:

Based on the accompanying table, the subject material is not a primary ocular irritant within the definition of the Act-Reference: Section 1500.42 (b) (1) (2) P. 27019 and requires no cautionary labeling with respect to that section.

Karl L. Gabriel

Karl L. Gabriel, V.M.D., Ph.D.
Director

TABLE I

Grades for Ocular Lesions

Material: 3M Company - T-2001CoC, as supplied.

	Rabbit No.	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
1 hour	1	0	0	0	1	1	3
	2	0	0	0	1	2	3
	3	0	0	0	1	1	3
	4	0	0	0	1	2	3
	5	0	0	0	2	2	2
	6	0	0	0	1	2	3
24 hours	1	0	0	0	0	0	0
	2	0	0	0	1	1	1
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
48 hours	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
72 hours	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
5 days	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
7 days	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0

FC-128

BIOSEARCH INC. p.o. box 8598 philadelphia, pennsylvania 19101
telephone: (215) 848-4499
Project Number - 77-1127A

Submitted to:

3M Company
3M Center
St. Paul, Minnesota
55101

Material: 3M Company - T-2001CoC

Sample Received: 11/21/77 Study Initiated: 11/22/77 Study Completed: 11/25/77

Date of Report: 1/6/78

Test: Primary Skin Irritation Study - Rabbits

Object of Test: To determine the degree of irritation, if any, which the subject material may produce when applied to the intact and abraded skin of albino rabbits.

Method of Test: The method employed in the testing, evaluation and the scoring of the results was similar to that described in Section 1500.41 - Hazardous Substances and Articles, Administration and Enforcement Regulations, Federal Register, Vol. 38, No. 187, P. 27019, 27 September 1973.

In carrying out the study the experimental sample was used as supplied. A group of six albino rabbits were clipped over a wide area. One side of the animals' backs was abraded at one site with a lancet sufficiently deep to penetrate the stratum corneum but not enter the derma to produce bleeding. The skin of the other side was allowed to remain intact. A 0.5 ml portion of material was applied to an abraded and an intact skin site on the same rabbit. Gauze patches were then placed over the treated areas and an impervious material was wrapped snugly around the trunks of the animals to hold the patches in place.

The wrapping was removed at the end of the twenty-four hour period and the treated areas were examined. Readings were also made after seventy-two hours. The Draize method of scoring was employed.

Results: See Table 1.

Conclusion: Based on the accompanying table, the subject material would not be classified as a primary irritant to albino rabbits within the definition of the Act-Reference: Section 1500.3 (c) (4) and requires no cautionary labeling with respect to that section.

Karl L. Gabriel

Karl L. Gabriel, V.M.D., Ph.D.
Director

TABLE 1

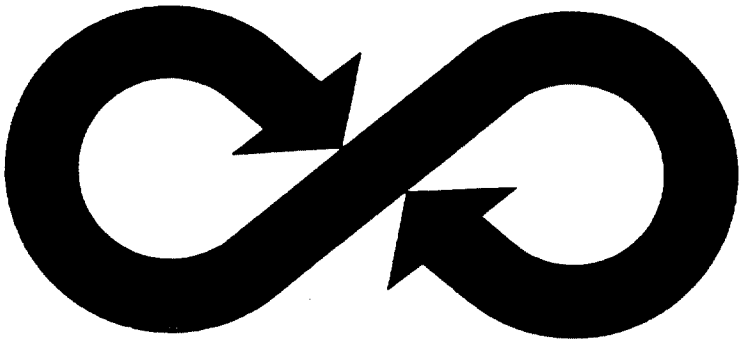
Primary Skin Irritation

Material: 3M Company - T-2001CoC, as supplied.

Erythema and Eschar Formation	Reading (Hours)	Rabbit Number 1	2	3	4	5	6	Average
Intact Skin	24	0	0	0	0	0	0	0.00
Intact Skin	72	0	0	0	0	0	0	0.00
Abraded Skin	24	0	0	0	0	0	0	0.00
Abraded Skin	72	0	0	0	0	0	0	0.00
				Subtotal				0.00
Edema Formation								
Intact Skin	24	0	0	0	0	0	0	0.00
Intact Skin	72	0	0	0	0	0	0	0.00
Abraded Skin	24	0	0	0	0	0	0	0.00
Abraded Skin	72	0	0	0	0	0	0	0.00
				Subtotal				0.00
				Total				0.00

Primary Irritation Score: 0

FC-128



Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 78-7186

AN ACUTE INHALATION TOXICITY STUDY OF T-2307 CoC IN THE RAT

Submitted to: Minnesota Mining and Manufacturing Company
St. Paul, Minnesota 55101

Attention: James E. Long, Sc.D.

Date: February 8, 1979

I. GENERAL

An experiment was performed to assess the acute inhalation toxicity of a dust of T-2307 CoC in Sprague-Dawley rats. The test material, received from the Minnesota Mining and Manufacturing Company, was labeled "3M Company, T2307CoC," and was in the form of a fine, yellow-orange powder.

II. EXPERIMENTAL

Two test material exposures were performed. For the first exposure, the test material was placed in a 500-milliliter, three-neck flask, fitted with a stir bar. For the second exposure, the test material was sieved through a 60-mesh sieve and placed in a 1000-milliliter, three-neck flask, fitted with a stir bar. The flasks were place on a magnetic stir plate to provide constant agitation of the test material during the exposure periods. Dry air, at the flow rate of 10 liters per minute, was passed through the test material, and the resulting dust-laden airstream was directed into a 26.5-liter glass exposure chamber containing the test animals. The exposures lasted for one hour. The flasks containing the test material were weighed before and after the respective exposure periods. The differences in weight were equal to the amounts of material consumed during the exposures. The nominal concentrations were calculated by dividing the weight lost by the total air flow through the chambers during the exposures.

II. EXPERIMENTAL (cont.)

The test animals consisted of two groups of five male and five female Sprague-Dawley rats obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. On the days of exposure (Day 0 - 10/4/78 and 10/12/78), the pre-exposure weights ranged from 200 to 297 grams. The basic health status of the test animals was established by a pre-exposure examination. The animals were observed for abnormalities at 15-minute intervals during the exposure period, upon removal from the chamber, hourly for four hours post-exposure, and daily thereafter for 14 days. Individual body weights were recorded prior to exposure on Day 0 and on Day 1, Day 2 (Group I only), Day 3 (Group II only), Day 4, Day 7 and Day 14 (terminus). On Day 14, all survivors were sacrificed (ethyl ether) and gross necropsy examinations were performed. All animals dying spontaneously were examined by gross necropsy as soon as possible after death.

III. RESULTS AND DISCUSSION

During the first exposure period (Group I), a total of 39.98 grams of the test material was delivered in a total volume of 600 liters of dry air, yielding a nominal exposure concentration of 66.63 milligrams per liter. During the second exposure period (Group II), 13.33 grams of the test material was delivered in the same volume of dry air, yielding a nominal exposure concentration of 22.22 milligrams per liter.

III. RESULTS AND DISCUSSION (cont.)

After 32 minutes of the Group I exposure, the chamber atmosphere remained static for a short period of time (less than five minutes) while the delivery flask was refilled with test material.

Abnormalities noted in the Group I animals throughout the exposure period were mucoid nasal discharge, excessive lacrimation, and excessive salivation. Squinting or closing of the eyes was observed from 30 minutes of exposure through exposure termination. Chromodacryorrhea was observed in one animal at exposure termination. Signs observed in the Group I animals upon their removal from the exposure chamber were excessive lacrimation (eight of ten rats), excessive salivation (one of ten rats), chromodacryorrhea (two of ten rats), mucoid nasal discharge (three of ten rats), and dry rales (one of ten rats). These signs were also observed sporadically during the four hourly post-exposure observation intervals. Seven of ten rats from Group I died during the 14-day observation period. Pre-death signs in these animals were mucoid nasal discharge (five of seven rats), red nasal discharge (five of seven rats), dry rales (one of seven rats), excessive lacrimation (one of seven rats), excessive salivation (one of seven rats), labored breathing (five of seven rats), yellow staining of the ano-genital fur (seven of seven rats), brown staining of the ano-genital fur (one of seven rats), reduced activity (seven of seven rats), poor general condition (seven of seven rats), spasms (one of seven rats), coldness of the body (two of seven rats), brown nasal discharge (one of seven rats), hair loss (one of seven rats), and rapid breathing (one of seven rats). Signs observed in the three surviving animals during the 14-day in-life period were red nasal discharge (two of three rats), mucoid nasal discharge (three of three rats)

III. RESULTS AND DISCUSSION (cont.)

excessive lacrimation (two of three rats), dry rales (one of three rats), labored breathing (three of three rats), yellow staining of the ano-genital fur (three of three rats), brown staining of the ano-genital fur (two of three rats), reduced activity (three of three rats), poor condition (three of three rats), spasms (one of three rats), loss of righting reflex (one of three rats), rapid breathing (one of three rats), and pilo erection (one of three rats).

Individual body weights and necropsy observations of the Group I animals are presented in Table I. Animals dying during the study lost weight steadily prior to death. Two of three surviving rats also exhibited steady weight losses. One male rat (#14), however, was gaining weight by termination of the study. Necropsy observations of the Group I animals were liver discoloration (nine of ten rats), lung discoloration (six of ten rats), adrenal discoloration (one of ten rats), adrenal enlargement (two of ten rats), gaseous distention of the stomach (three of ten rats), gaseous distention of the intestines (one of ten rats), stomach discoloration (one of ten rats), and intestinal discoloration (two of ten rats).

Abnormalities noted in the Group II (22.22 mg/l) rats during the exposure period were excessive lacrimation, excessive salivation, squinting or closing of the eyes, mucoid or red nasal discharge, and/or labored breathing. Upon removal of the animals from the exposure chamber, dry rales (three of ten rats), mucoid nasal discharge (two of ten rats), excessive salivation (one of ten rats), excessive lacrimation (two of ten rats), and yellow staining of the ano-genital fur (nine of ten rats) were observed. These signs were also observed in the rats

III. RESULTS AND DISCUSSION (cont.)

during the four hourly post-exposure intervals. Other signs observed sporadically during those intervals were labored breathing and reduced activity. Observations made during the 14-day in-life period were dry rales (seven of ten rats), mucoid nasal discharge (seven of ten rats), rapid breathing (two of ten rats), yellow staining of the ano-genital fur (eight of ten rats), brown staining of the ano-genital fur (two of ten rats), orange staining of the ano-genital fur (two of ten rats), reduced activity (two of ten rats), pilo erection (one of ten rats), hair loss (one of ten rats), coldness of the body (two of ten rats), and generally poor condition (ten of ten rats). Individual body weights and necropsy observations of the Group II animals are presented in Table II. All Group II rats experienced weight loss following exposure to the test material. Though weight gains were retarded, all male rats exceeded their Day 0 body weights by termination of the study. None of the female rats recovered their original weights by Day 14, though three of five females were gaining weight by the end of the study. Necropsy observations of these animals revealed lung discoloration in eight of ten rats.

IV. CONCLUSION

A pair of exposures were performed on Sprague-Dawley rats to determine the acute inhalation toxicity of different concentrations of a dust of T-2307 CoC. The first exposure (Group I) yielded a nominal test material exposure concentration of 66.63 milligrams per liter. Seven of ten animals of this group were dead by Day 10 of the study. The second exposure (Group II) yielded a nominal exposure test material concentration of 22.22 milligrams per liter. There was no mortality in the second group. However, all animals exposed to the test material in this study displayed a definite response to their exposure to the dust of T-2307 CoC. In-life observations showed the animals to be in poor condition following the exposure and post-exposure weight gains were markedly depressed. Necropsy examination of the Group I animals revealed liver discoloration in nine of ten rats, marked lung discoloration in six of ten rats and adrenal enlargement in two of ten rats. The main findings on necropsy of Group II animals after 14 days was lung discoloration in eight of ten rats. These findings, especially in Group I, would appear to be indicative of a residual effect of the test material exposure.

George M. Rusch, Ph.D.
Director, Inhalation Technology

William E. Rinehart, Sc.D.
Vice President, Science

Written by: Ilona R. Jupina

/mes

Table I
An Acute Inhalation Toxicity Study
of T-2307 CoC in the Rat

Individual Body Weights and Necropsy Observations
Group I - 66.63 mg/l

Animal Number	Sex	Body Weights (g) Day 0	Day 1	Day 2	Day 4	Day 7	Day 14	Necropsy Observations*
10	M	248	221	207	195	Dead[b]		B. lungs red in color. Liver mottled tan and red. Stomach distended with gas.
11	M	270	242	226	209	Dead[a]		B. lungs red in color. Liver mottled tan and red.
12	M	297	273	260	241	245	216	All lobes of liver mottled tan and red.
13	M	269	251	247	228	197	Dead[c]	B. lungs red in color. All lobes of liver mottled red and grey.
14	M	297	271	267	257	202	236	All lobes of liver mottled red and tan.
20	F	208	198	194	173	149	Dead[c]	B. lungs red in color. All lobes of liver black-red with tan and grey patches. B. adrenal enlarged in appearance and red in color.
21	F	206	194	187	185	183	179	N.O.A.
22	F	234	207	199	184	172	Dead[d]	R. lobe of liver mottled red and tan. B. adrenals enlarged (1.5 x normal size) in appearance.
23	F	228	215	208	183	163	Dead[e]	B. lungs red in color. All lobes of liver abnormally dark red in color. Stomach distended with gas. Small intestines yellow in color, distended with gas.
24	F	238	220	205	194	175	Dead[e]	B. lungs red in color. All lobes of liver abnormally dark red in color. Stomach pale yellow in color, distended with gas. Small intestines yellow in color.

* N.O.A. - no observed abnormalities.
Key: R = right; L = left; B = bilateral.
a Spontaneous death Day 6.
b Spontaneous death Day 7.
c Spontaneous death Day 8.
d Spontaneous death Day 9.
e Spontaneous death Day 10.

Table II
An Acute Inhalation Toxicity Study
of T-2307 CoC in the Rat

78-7186

Individual Body Weights and Necropsy Observations
Group II - 22.22 mg/l

Animal Number	Sex	Body Weights (g) Day 0	Day 1	Day 3	Day 4	Day 7	Day 14	Necropsy Observations*
30	M	281	253	265	256	250	291	Scattered red foci on B. lungs.
31	M	288	266	255	258	272	301	B. lungs mottled pink and red with scattered grey foci.
32	M	266	238	234	237	246	272	B. lungs mottled tan and red with scattered grey foci.
33	M	276	267	262	260	265	282	B. lungs mottled pink and tan with scattered grey foci.
34	M	282	275	277	274	284	314	Scattered red and grey foci on B. lungs.
40	F	214	207	200	194	189	202	Scattered red foci on B. lungs.
41	F	206	179	175	175	177	169	B. lungs mottled pink and red.
42	F	200	196	196	188	188	187	N.O.A.
43	F	224	219	222	210	220	220	N.O.A.
44	F	207	182	179	175	164	195	B. lungs mottled pink and tan.

* N.O.A. - no observed abnormalities.

Key: R = right; L = left; B = bilateral.

STUDY CODE : K01-1815

(写)

[redacted]

FINAL REPORT

BACTERIAL REVERSE MUTATION TEST
OF
υ -1

October, 1996

Hita Research Laboratories
Chemical Biotesting Center
Chemicals Inspection & Testing Institute
Japan

QUALITY ASSURANCE STATEMENT

Hita Research Laboratories, Chemical Biotesting Center
Chemicals Inspection & Testing Institute, Japan

Sponsor: SUMITOMO 3M LIMITED
Title: Bacterial reverse mutation test of υ-1
Study code: K01-1815

This report was audited by the Quality Assurance Section.
I, the undersigned, hereby declare that this report reflects
the original Japanese report.

(Date) December 17, 1996

(Signature)

Section Chief, Quality Assurance

Keiji Shiraishi, B.S.

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report.
(Study code No. K01-1815 issued on October 30, 1996)

(date) December 17, 1996

(signature) Shozo Ogura

Shozo Ogura
Hita Research Laboratories
Chemical Biotesting Center
Chemicals Inspection &
Testing Institute, Japan

GLP STATEMENT

Hita Research Laboratories, Chemical Biotesting Center
Chemicals Inspection & Testing Institute, Japan

Sponsor: SUMITOMO 3M LIMITED
Title: Bacterial reverse mutation test of v-1
Study Code No.: K01-1815

I, the undersigned, hereby declare that this study was conducted in compliances with "Standards to be observed by Testing Institutions for Toxicity Investigations" (Japan's MOL, No.76, September 1, 1988).

Management: Signed in original October 30, 1996
Shigetaka Yamane, Ph. D.

QUALITY ASSURANCE STATEMENT

Hita Research Laboratories, Chemical Biotesting Center
Chemicals Inspection & Testing Institute, Japan

Sponsor: SUMITOMO 3M LIMITED
Title: Bacterial reverse mutation test of υ-1
Study Code No.: K01-1815

This study was audited by the Quality Assurance Section and the study procedures were inspected on the following dates.

Dates of Inspections and Audits	Dates of Reports to Study Director	Dates of Reports to Management
September 12, 1996	September 13, 1996	September 17, 1996
October 1, 1996	October 1, 1996	October 1, 1996
October 30, 1996	October 30, 1996	October 30, 1996

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect the raw data obtained.

Section Chief, Quality Assurance: Signed in original October 30, 1996
Keiji Shiraishi, B.S.

Study code: K01-1815
Test substance code: HR3291
Sponsor code: S-030

TITLE

Bacterial reverse mutation test of υ -1

SPONSOR

SUMITOMO 3M LIMITED

8-8, Minami-Hashimoto 3-chome Sagamihara-shi, Kanagawa, 229 Japan

TESTING FACILITY

Hita Research Laboratories, Chemical Biotesting Center

Chemicals Inspection & Testing Institute, Japan

822, 3-chome, Ishii-machi, Hita, Oita 877, Japan

PURPOSE OF STUDY

The purpose of this study was to determine the mutagenic potential of the test substance using *Salmonella typhimurium* and *Escherichia coli*.

TESTING METHOD

This study was conducted in accordance with the following guidelines: "Standards for Toxicity Investigations" (Japan's MOL, No.77, September 1, 1988).

GLP COMPLIANCE

This study was carried out in compliance with the following GLP requirement: "Standards to be observed by Testing Institutions for Toxicity Investigations" (Japan's MOL, No.76, September 1, 1988).

PERIOD OF STUDY

Commencement of test:	September 17, 1996
Dose finding test:	September 25, 1996
Completion of observation:	October 14, 1996
Presentation of final report:	October 30, 1996

LOCATION AND PERIOD FOR RETENTION OF RAW DATA

Data and test substance are retained in the archives and the test substance storage room of Hita Research Laboratories for 10 years following the date of the notification specified under Item 1 of Article 57-2 of Industrial Safety & Health Law, respectively.

After termination of the retention period, any measures taken are done so with the approval of the sponsor.

PERSON CONCERNED WITH STUDY

Study Director:

Signed in original October 30, 1996
Shozo Ogura
Hita Research Laboratories
Mutagenicity Section

Study Staff: Tsunehiko Inai, B.S.

Person in charge of Storage: Shizuka Kouda

ANY UNEXPECTED SITUATIONS AND DEVIATIONS FROM PROTOCOL

There were no unexpected situations and deviations from protocol which might have affected the test results.

CONTENTS

SUMMARY

The reverse mutation test of υ-1 was performed on *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537 and a *Escherichia coli* strain WP2 *uvrA* using the pre-incubation method with and without metabolic activation.

The results showed that the numbers of their revertant colonies for all strains in groups which were treated with the test substance were less than twice that of each negative control with and without S9 Mix.

The numbers of the revertant colonies in the negative control and the positive controls were within the background data in our laboratories.

Based upon the above results, υ-1 was judged to have no reverse mutagenic potential under the present test conditions.

MATERIALS AND METHODS

1. TEST SUBSTANCE AND POSITIVE CONTROLS

1.1 Test substance (Information provided by the sponsor)

1) Name

Potassium salt of N-ethyl-N-perfluorobutylsulfonylglycine

Other name: υ -1

CAS No.: 67584-51-4

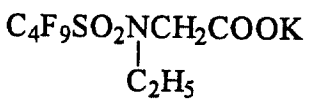
2) Lot No.

Lot 1

3) Supplier

SUMITOMO 3M LIMITED

4) Structural formula or rational formula (Outline of manufacturing method, in case both were unknown)



(molecular formula $C_8H_7F_9KNO_4S$)

5) Purity

97.3 w/w%

6) Impurities

KCl 2.7 w/w%

7) Physicochemical properties

Appearance at ordinary temperature:	light gray powder
Molecular weight:	423.30
Stability:	stable
Melting point:	—
Boiling point:	—
Vapor pressure:	—
Partition coefficient:	—
Solubility:	—
Degree of solubility:	Water: ≧5 w/v%*
	DMSO: ≧5 w/v%*
	Acetone: < 10 w/v%*
	Others: —

* Examined in our laboratories

8) Storage conditions

room temperature

9) Care on handling

Gloves, a mask, a head cap and a lab coat were worn when handling.

1.2 Positive controls

1) 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)

Manufacturer:	Wako Pure Chemical Industries, Ltd.
Lot No.:	LEN0571
Properties:	reddish-orange crystalline powder
Purity:	99.5%
Grade:	special grade

2) Sodium azide (NaN_3)

Manufacturer:	Wako Pure Chemical Industries, Ltd.
Lot No.:	DLP2438
Properties:	white crystalline
Purity:	99.4%
Grade:	special grade

3) 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine · 2HCl (ICR-191)

Manufacturer:	Polysciences, Inc.
Lot No.:	412795
Properties:	yellow crystalline powder
Purity:	—
Grade:	—

4) 2-Aminoanthracene (2AA)

Manufacturer:	Wako Pure Chemical Industries, Ltd.
Lot No.:	DLR7869
Properties:	yellowish-green-brown powder
Purity:	95.7%
Grade:	—

5) Storage conditions

A cold and dark place

6) Care on handling

Gloves, a mask, a head cap and a lab coat were worn when handling.

2. BACTERIAL STRAINS

2.1 Strains selected

Salmonella typhimurium strains TA100, TA98, TA1535 and TA1537 were obtained from Dr. B.N. Ames, University of California, U.S.A. ,on June 20, 1990.

A *Escherichia coli* strain WP2 *uvrA* was obtained from Japan Bioassay Laboratories, on April 6, 1995.

S. typhimurium strains TA100, TA1535 and a *E. coli* strain WP2 *uvrA* were used for the detection of base-pair substitution mutation, while *S. typhimurium* strains TA98 and TA1537 were for the detection of frameshift mutation.

2.2 Storage

The test strains were stored as frozen stock cultures (0.045 ml of dimethyl sulfoxide (DMSO)*/0.5 ml of broth culture) at –80℃ (ultra-deep freezer MDF-291, Sanyo).

* Purity ≧ 99.0%, Lot No. CF103, Dojindo Laboratories

2.3 Characterization of strains

1) Characteristics of strains

Strains	Mutation on synthesis of amino acid	Mutation on excision repair	Membrane mutation (LPS)	R-factor (pKM101)
Salmonella typhimurium				
TA1535	*hisG46*	△*uvrB*	*rfa*	–
TA1537	*hisC3076*	△*uvrB*	*rfa*	–
TA98	*hisD3052*	△*uvrB*	*rfa*	+
TA100	*hisG46*	△*uvrB*	*rfa*	+
Escherichia coli				
WP2 *uvrA*	*trp*	△*uvrA*	+	–

The amino acid requirement for growth was demonstrated by using histidine for *S. typhimurium* strains and tryptophan for *E. coli* strain. The presence of R-factor, membrane mutation and mutation on the ability to repair DNA lesions were confirmed by ampicillin resistance, sensitivity to crystal violet and UV sensitivity, respectively.

2) Date of characterization

Salmonella typhimurium	TA1535	July 18, 1996
	TA1537	June 7, 1996
	TA98	June 7, 1996
	TA100	March 6, 1996
Escherichia coli	WP2 *uvrA*	April 18, 1996

3. MEDIUM AND S9 MIX

3.1 Medium

1) Minimal glucose agar plate (prepared in our Laboratories)

The medium was prepared as follows, and poured 30 ml into a petri dish.

Components	Amount included in one litre
20 × Vogel-Bonner E	50 ml
40 w/v% Glucose	50 ml
Agar	15 g

(1) Agar: Bacto-Agar (Lot No. 71892AJB or 90800JA, Difco Laboratories)

(2) Manufacturing date: dose finding test on September 11, 1996
main test on October 3, 1996

2) Soft agar

The solution containing 0.5 mM histidine and 0.5 mM biotin for *S. typhimurium* strains or 0.5 mM tryptophan for *E. coli* strain was added to the soft agar solution containing 0.6 w/v% agar (Bacto-Agar, Lot No. 71892AJB, Difco Laboratories) and 0.5 w/v% NaCl in a ratio of 1 : 10.

3.2 S9 Mix

1) Rat liver S9 (Kikkoman Co., Ltd.)

Induction method: SD male rats, 7-week-old (203-254 g), were intraperitoneally administrated phenobarbital (30 mg/kg × 1 time, 60 mg/kg × 3 times) and 5,6-benzoflavone (80 mg/kg × 1 time).

Lot No.: RAA-350 (manufactured on August 23, 1996, purchased on September 4, 1996)

Storage: -80℃ (ultra-deep freezer MDF-291, Sanyo)

2) Cofactor for S9 Mix (Oriental Yeast Industries, Ltd.)

Lot No.: 999602

Storage: -20℃ (bio-freezer GS-2603, Nippon Freezer Ltd.)

3) Composition of S9 Mix

One ml of S9 Mix contained 8 μmol $MgCl_2$, 33 μmol KCl, 5 μmol G-6-P, 4 μmol NADPH, 4μmol NADH, 100 μmol of 0.2 M sodium-phosphate buffer (pH 7.4) and 0.1 ml S9.

4. PRE-CULTURES

From the stock cultures, 20 μl of the bacterial suspension was inoculated to L-tube containing 10 ml nutrient broth No.2 (Lot No. 194 56443, OXOID Ltd.) and the bacterial culture was incubated at 37 ± 0.5℃ for 8 h with shaking at 50 times/min by the Monod shaker (MONOSIN- II A, Taitec Co., Ltd.) The viable cell counts calculated from the values which were determined at 660 nm by spectrophotometry (Novaspec, LKB Japan) at the end of incubation are shown below.

		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
No. of viable cells ($\times 10^9$/ml)	Dose finding test	2.1	2.1	4.4	2.4	2.1
	Main test	2.1	2.1	4.0	2.3	2.0

5. PREPARATION OF TEST SUBSTANCE AND POSITIVE CONTROLS

5.1 Test substance

1) Preparation

The test substance was dissolved in distilled water (distilled water for injection, Lot No. K6B74, Otsuka Pharmaceutical Factory) to make 5 w/v% concentration and diluted with the same solvent to give appropriate concentrations.

2) Stability of the test solution

No denaturation of the test solution was observed for the color and the exothermic reaction until 2 hours after preparation.

3) Preparation time

Prepared immediately before use and used within 0.5 h at room temperature.

5.2 Positive controls

1) Preparation

NaN_3 was dissolved in distilled water (Lot No. K6B74). AF-2, ICR-191 and 2AA were dissolved in DMSO (Lot No. CD069).

2) Preparation time and storage condition

Prepared on every 3 months and stored at -80℃ (ultra-deep freezer MDF-291, Sanyo).

6. METHODS

The test was carried out for *S. typhimurium* strains TA1535, TA1537, TA98, TA100 and a *E. coli* strain WP2 *uvrA* using the pre-incubation method both with and without metabolic activation system. The plating was done in triplicate for the negative control and in duplicate for the test substance and positive controls.

6.1 Procedures

After 0.1 ml of the test substance solution, 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4) or S9 Mix, and 0.1 ml of the bacterial culture were added to a tube, the mixtures were incubated for 20 min at 37 ± 0.5℃. Two ml of the soft agar was then added to each tube and poured onto a minimal glucose agar plate.

After incubation for 48 h at 37 ± 0.5℃, the number of revertant colonies were counted.

As the sterility test, each 0.1 ml of each bacterial suspension, test substance solution, S9 Mix or 0.1 M sodium phosphate buffer (pH 7.4) were smeared on a minimal glucose agar plate and incubated at 37 ± 0.5℃ for 48 h, and then checked the bacterial contamination. Distilled water was used as a negative control, and the following positive controls were used for each bacterial strains.

	TA100	TA1535	WP2 *uvrA*	TA98	TA1537
S9 Mix (–)	AF-2	NaN_3	AF-2	AF-2	ICR-191
	0.01	0.5	0.01	0.1	1
S9 Mix (+)	2AA	2AA	2AA	2AA	2AA
	1	2	10	0.5	2

(μg/plate)

6.2 Dose selection

1) Dose finding test

The test was carried out at the highest dose of 5,000 μg/plate and 6 doses of 1,000, 500, 100, 50, 10 and 5 μg/plate.

As a result, growth inhibition was observed at 5,000 μg/plate both with and without S9 Mix.

2) Main test

Based on the results of the dose finding test, a main test was performed at the highest dose of 5,000 μg/plate and 5 lower doses diluted with a geometric progression of 2.

7. MICROSCOPIC OBSERVATION AND COLONY COUNTING

7.1 Microscopic observation

The state of revertant colonies (size and number of colonies), deposition of the test substance and the growth inhibition were examined with a stereo microscope.

7.2 Colony counting

The number of colonies were counted with a manual counter or a colony analyzer (CA-7 or CA-9, Toyo-sokki Co., Ltd). Correction for counting errors was made for measurements with the colony analyzer. Each plate was measured three times, and the average of these three measurements was adopted as the number of revertant colonies on the plate. The average for each dose was calculated from the values of the plates used. Decimals of the average figures were rounded off.

8. INTERPRETATION OF RESULTS

The test substance was judged to be positive, when the number of revertant colonies was twice or more of the negative control, and when the dose-relationship and the reproducibility were obtained. Any statistical procedures were not used.

RESULTS

The numbers of their revertant colonies for all strains in groups which were treated with the test substance were less than twice that of each negative control with and without S9 Mix.

The positive controls showed the distinct increase of revertant colonies, and the positive controls and the negative control were within a range of the background data in our laboratories.

The growth inhibition was observed at more than 2,500 μg/plate both with and without S9 Mix.

There were no fluctuations which affected the test results since the sterility test confirmed the absence of any micro-organisms.

CONCLUSION

In conclusion, v -1 was judged to have no reverse mutagenic potential under the present test conditions.

REFERENCES

1. Ministry of Labor (1991) Guidebook on Mutagenicity Tests using Micro-organisms, New Edition (in Japanese) published by Japan Industrial Safety and Health Association,

2. Green M.H.L. and W.J. Muriel (1976) Mutagen testing using Trp^+ reversion in *Escherichia coli*, Mutation Res., 38: 3-32.

3. Maron, D.M., and B.N. Ames (1983) Revised methods for the *Salmonella* mutagenicity test, Mutation Res., 113: 173-215.

Dose finding test

Test substance: $\upsilon - 1$

With(+) or without(-) S9 Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2 *uvrA*	TA 98	TA 1537
S9 Mix (-)	negative control	90 101 (101) 111	13 10 (12) 14	33 41 (37) 37	30 33 (31) 31	9 6 (7) 7
	5	118 109 (114)	11 14 (13)	34 45 (40)	33 25 (29)	8 9 (9)
	10	99 126 (113)	11 12 (12)	39 44 (42)	19 27 (23)	9 5 (7)
	50	101 107 (104)	18 8 (13)	39 32 (36)	23 26 (25)	12 9 (11)
	100	98 116 (107)	12 16 (14)	33 34 (34)	31 36 (34)	10 10 (10)
	500	109 118 (114)	16 7 (12)	48 38 (43)	23 25 (24)	8 9 (9)
	1000	104 95 (100)	8 15 (12)	30 38 (34)	27 27 (27)	7 10 (9)
	5000	80* 76* (78*)	3* 5* (4*)	33* 20* (27*)	10* 15* (13*)	5* 8* (7*)
S9 Mix (+)	negative control	110 85 (95) 91	10 15 (12) 12	41 40 (38) 32	34 36 (34) 33	23 18 (19) 16
	5	91 96 (94)	8 12 (10)	37 39 (38)	34 34 (34)	15 17 (16)
	10	104 99 (102)	11 14 (13)	35 40 (38)	30 34 (32)	25 23 (24)
	50	101 90 (96)	12 8 (10)	35 39 (37)	31 23 (27)	17 14 (16)
	100	105 91 (98)	15 8 (12)	33 35 (34)	43 45 (44)	20 23 (22)
	500	100 96 (98)	12 7 (10)	36 41 (39)	30 43 (37)	16 21 (19)
	1000	93 110 (102)	9 12 (11)	32 32 (32)	31 39 (35)	19 20 (20)
	5000	120* 102* (111*)	6* 10* (8*)	25* 28* (27*)	25* 23* (24*)	0* 14* (7*)
Positive control not requiring S9 Mix	Name	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1
	Number of colonies/plate	351 410 (381)	382 385 (384)	179 188 (184)	487 566 (527)	1734 1968 (1851)
Positive control requiring S9 Mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	10	0.5	2
	Number of colonies/plate	727 621 (674)	133 158 (146)	650 644 (647)	304 274 (289)	149 163 (156)

Notes Parenthesis shows the mean of each plate.
* : Observed bacterial growth inhibition.

·AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

·NaN_3: Sodium azide

·ICR-191: 2-Methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine·2HCl

·2AA: 2-Aminoanthracene

Test substance: υ－1

With(+) or without(-) S9 Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2 *uvrA*	TA 98	TA 1537
S9 Mix (-)	negative control	113 112 (110) 106	18 10 (15) 17	39 36 (37) 36	28 25 (30) 38	13 10 (14) 19
	156	100 117 (109)	12 13 (13)	27 33 (30)	28 34 (31)	8 14 (11)
	313	132 100 (116)	11 15 (13)	24 33 (29)	30 34 (32)	20 8 (14)
	625	119 114 (117)	10 11 (11)	30 32 (31)	33 34 (34)	18 24 (21)
	1250	104 103 (104)	12 12 (12)	36 50 (43)	30 29 (30)	18 27 (23)
	2500	82* 91*(87*)	7* 8*(8*)	35* 30*(33*)	21* 23*(22*)	3* 9*(6*)
	5000	79* 88*(84*)	0* 0*(0*)	36* 33*(35*)	18* 24*(21*)	0* 6*(3*)
S9 Mix (+)	negative control	108 117 (115) 120	9 9 (11) 14	38 29 (34) 34	42 46 (42) 39	24 28 (26) 26
	156	103 116 (110)	6 8 (7)	49 38 (44)	35 40 (38)	23 35 (29)
	313	97 89 (93)	9 10 (10)	52 42 (47)	35 46 (41)	31 26 (29)
	625	109 115 (112)	11 13 (12)	35 36 (36)	31 37 (34)	26 26 (26)
	1250	110 112 (111)	13 7 (10)	38 53 (46)	41 45 (43)	26 32 (29)
	2500	106* 136*(121*)	8* 6*(7*)	33* 38*(36*)	37* 31*(34*)	7* 14*(11*)
	5000	103* 118*(111*)	3* 1*(2*)	41* 33*(37*)	24* 24*(24*)	0* 7*(4*)
Positive control not requiring S9 Mix	Name	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1
	Number of colonies/plate	356 337 (347)	328 295 (312)	133 113 (123)	456 470 (463)	2055 2081 (2068)
Positive control requiring S9 Mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	10	0.5	2
	Number of colonies/plate	804 874 (839)	191 163 (177)	582 561 (572)	276 255 (266)	171 159 (165)

Notes Parenthesis shows the mean of each plate.
* : Observed bacterial growth inhibition.

·AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

·NaN_3: Sodium azide

·ICR-191: 2-Methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine·2HCl

·2AA: 2-Aminoanthracene

Dose finding test

K01-1815

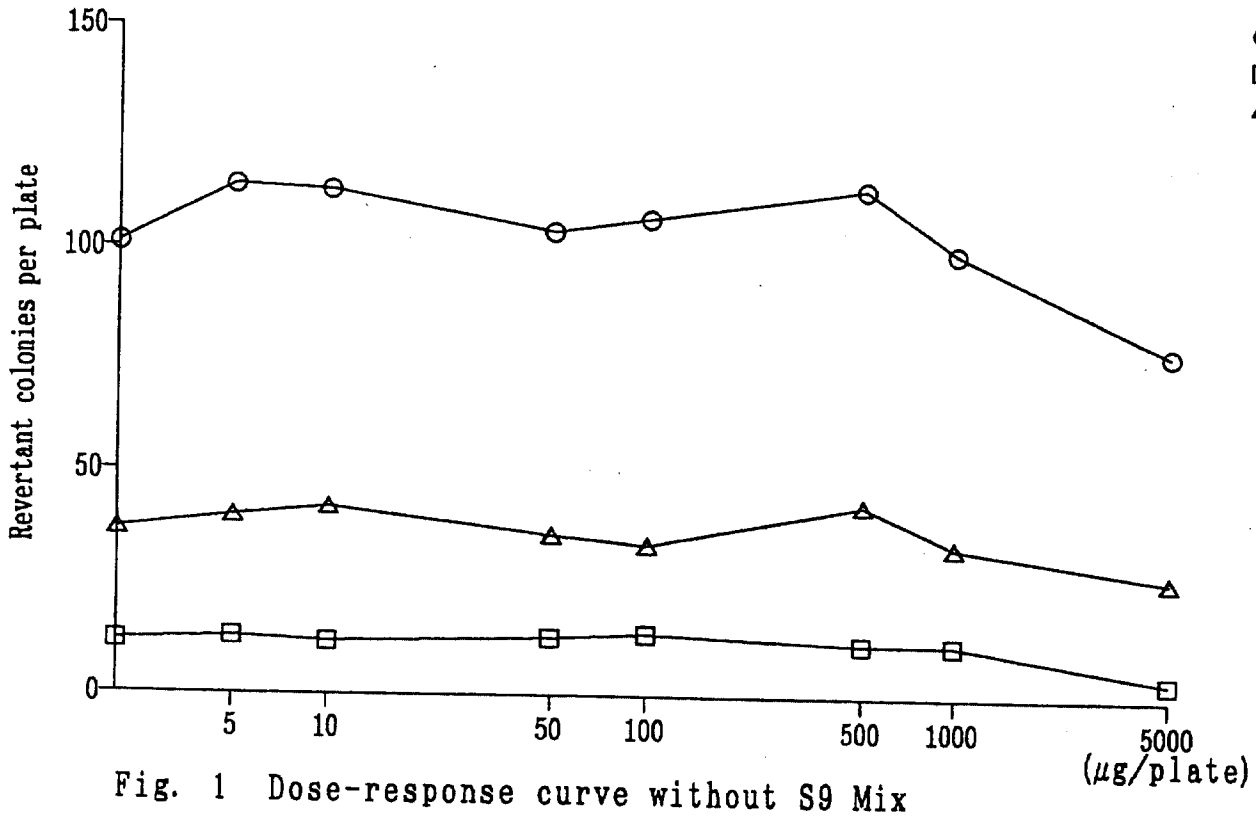


Fig. 1 Dose-response curve without S9 Mix

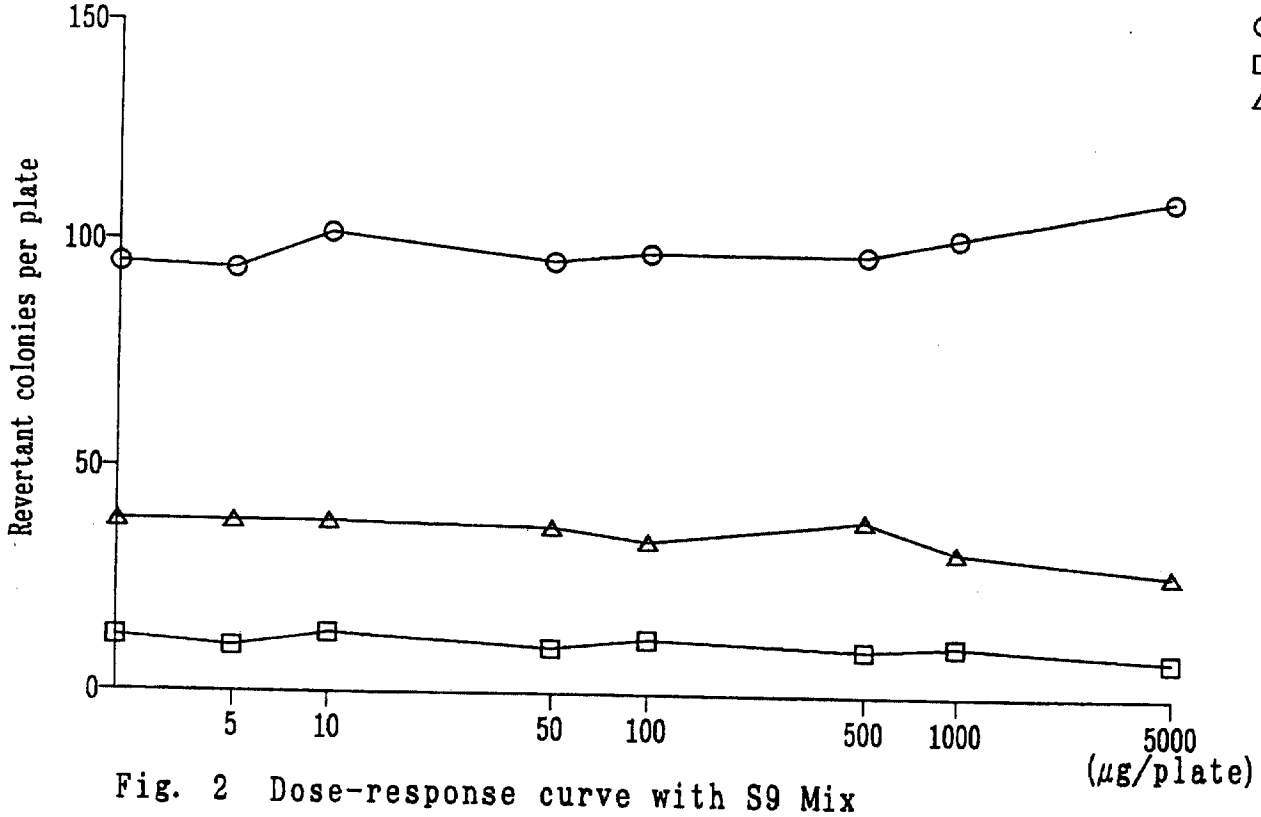


Fig. 2 Dose-response curve with S9 Mix

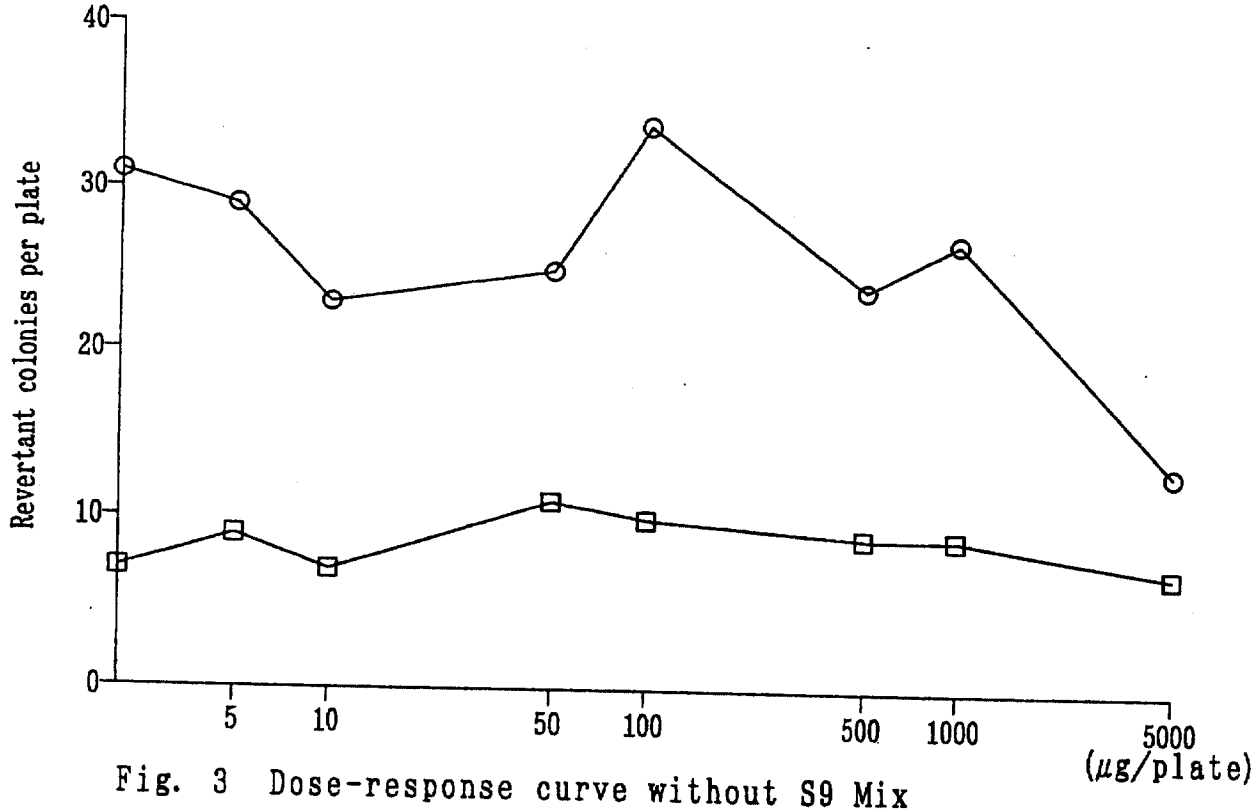


Fig. 3 Dose-response curve without S9 Mix

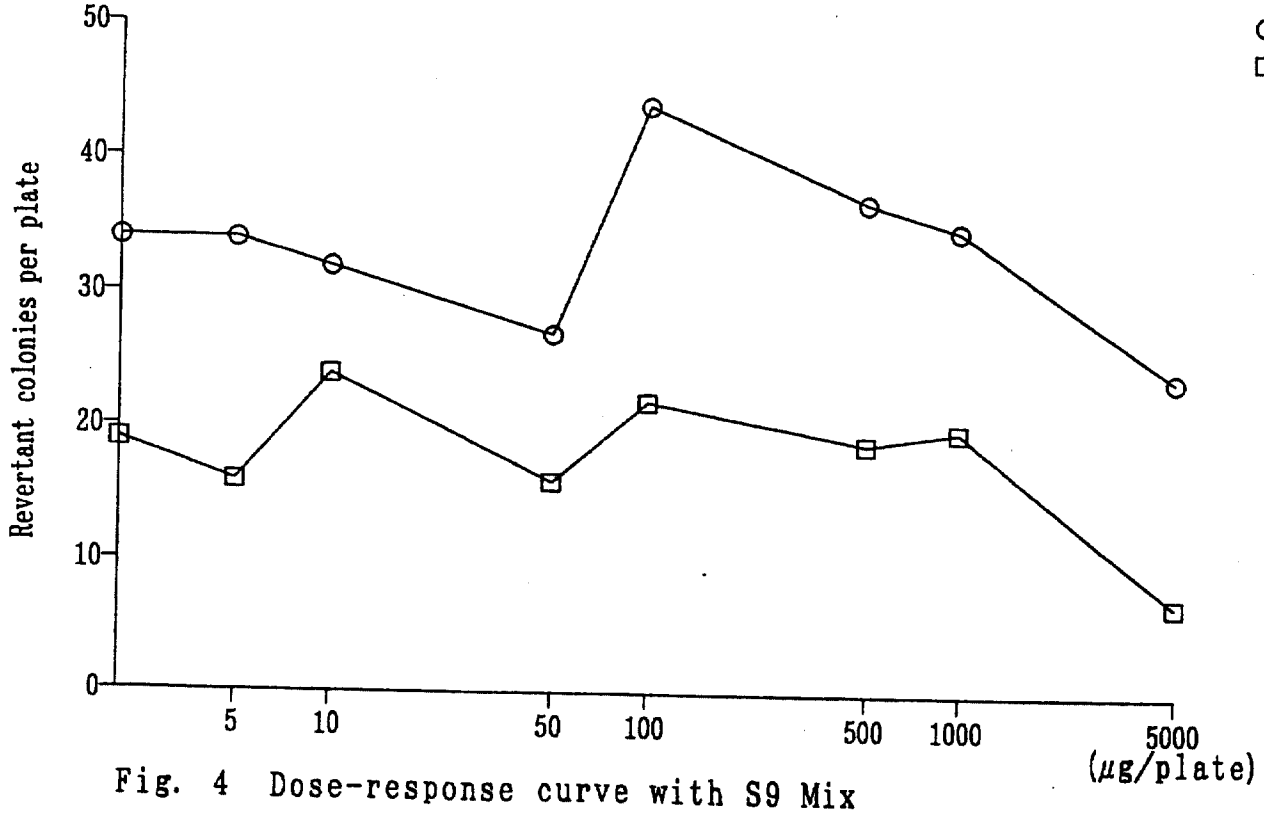


Fig. 4 Dose-response curve with S9 Mix

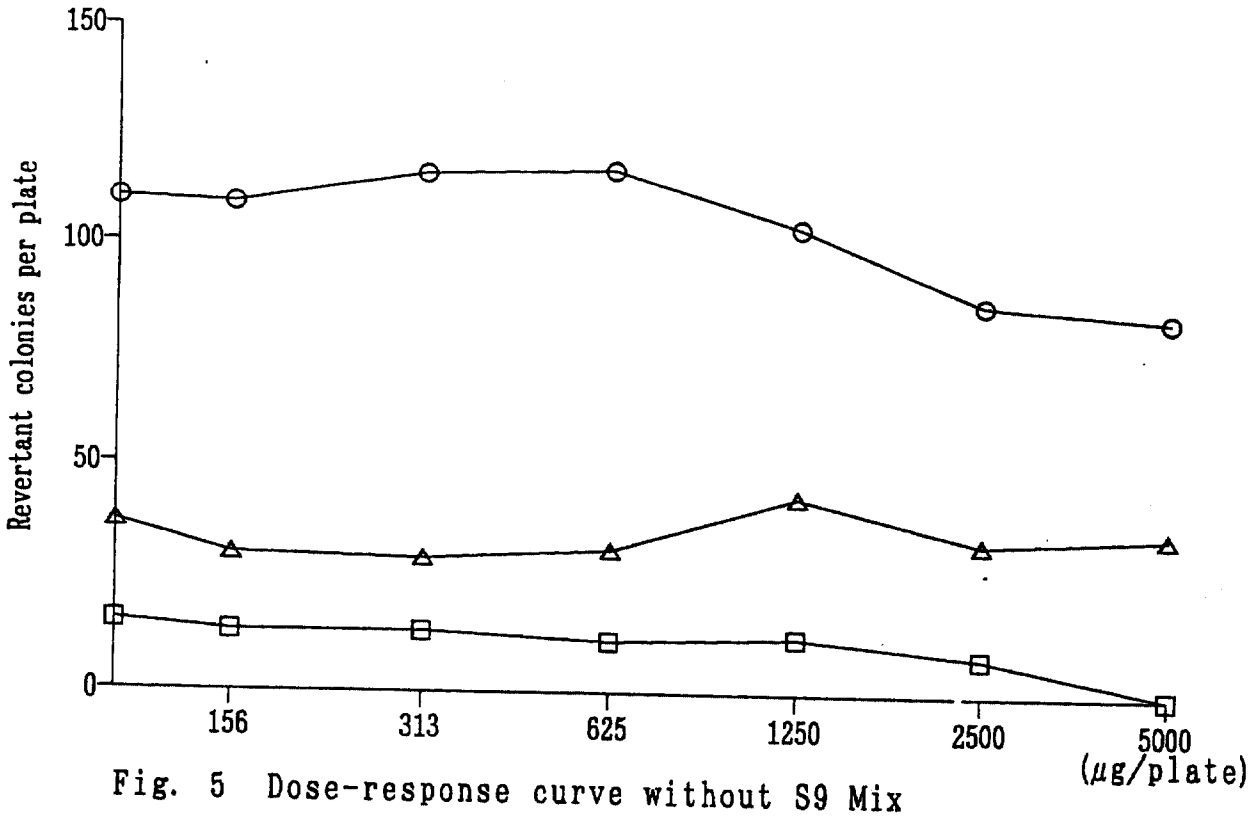


Fig. 5 Dose-response curve without S9 Mix

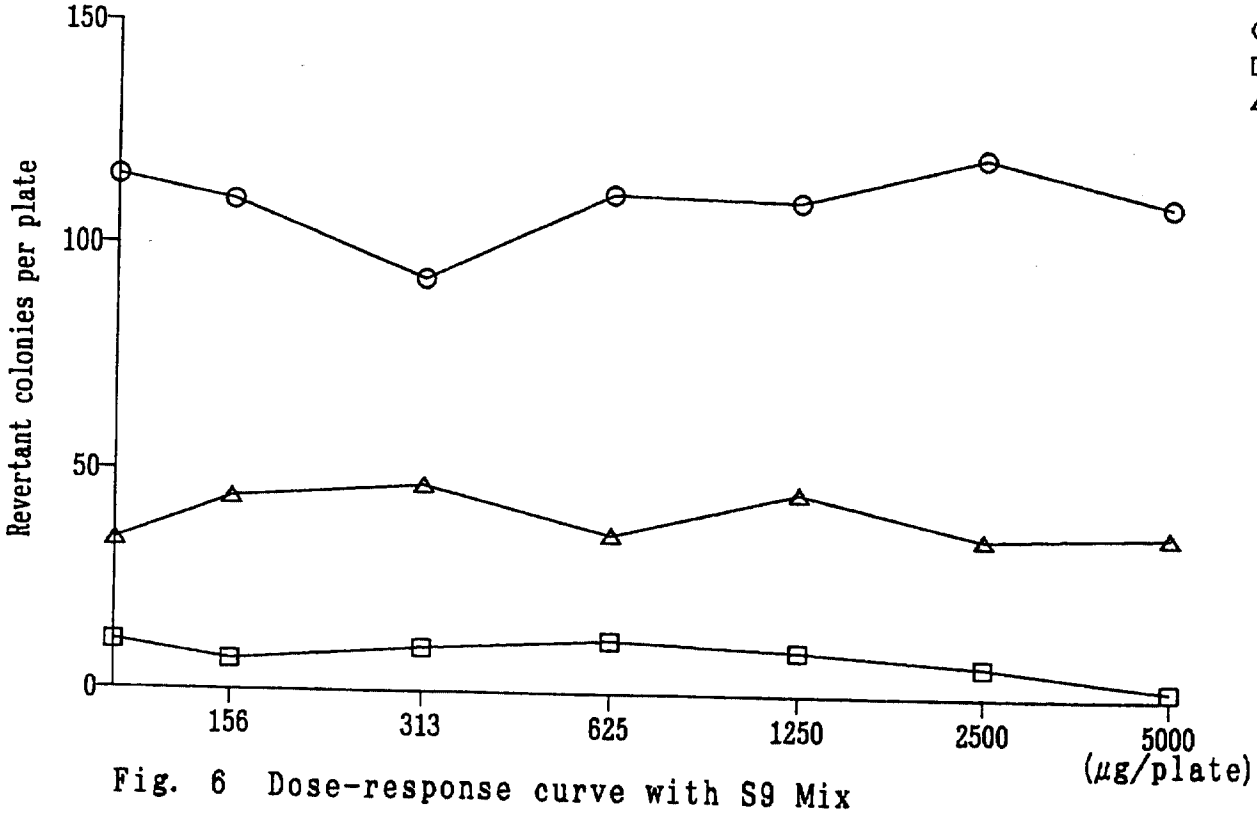


Fig. 6 Dose-response curve with S9 Mix

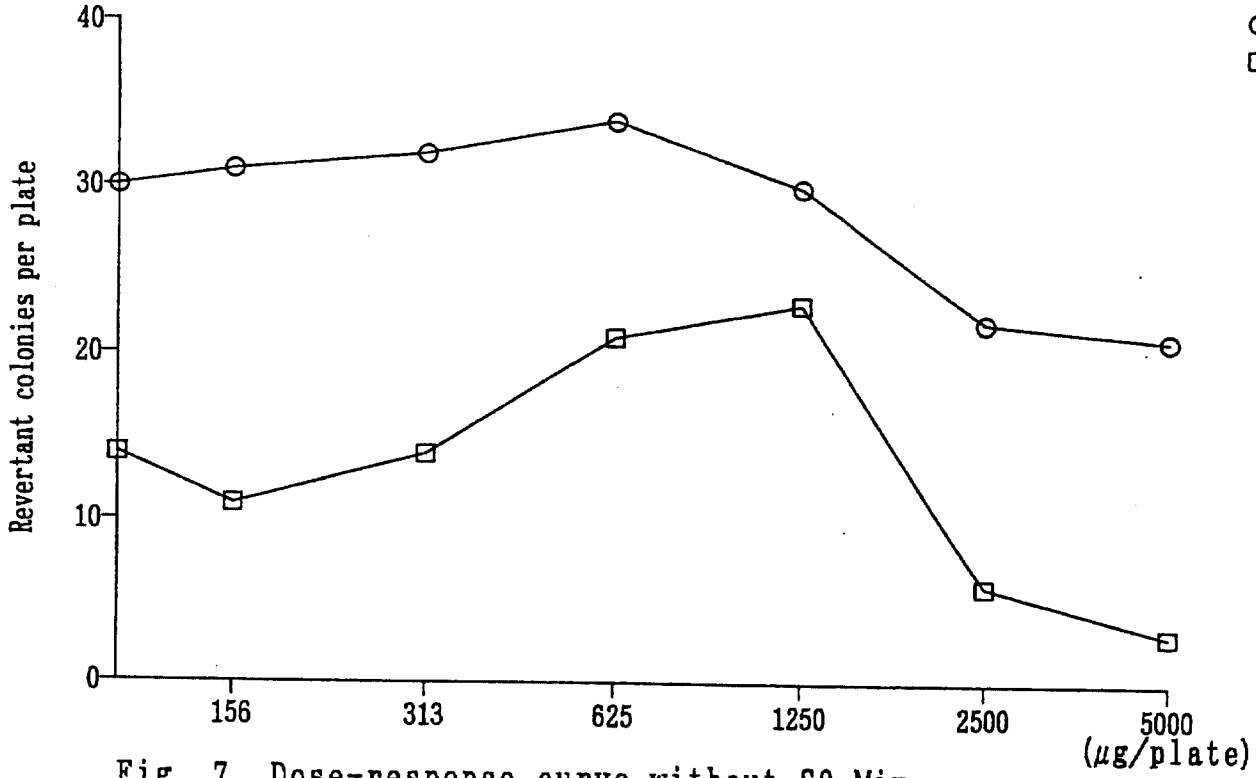


Fig. 7 Dose-response curve without S9 Mix

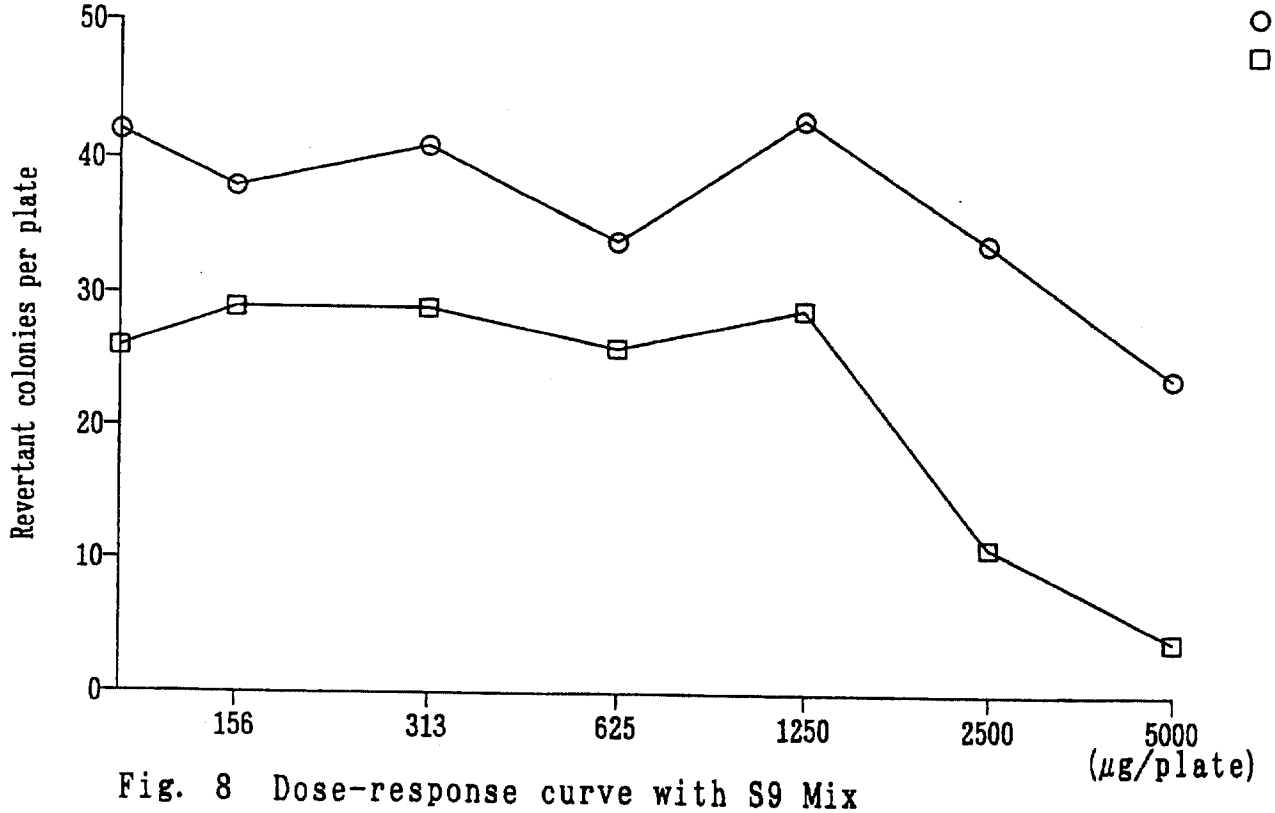


Fig. 8 Dose-response curve with S9 Mix

L-6778, F-6873

SRI International

IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY'S COMPOUND T-3290CoC

Final Report

November 1982

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SUMMARY

SRI International examined 3M Company's Compound T-3290CoC for mutagenic activity with strains TA1535, TA1537, TA1538, TA98, and TA100 of bacterium Salmonella typhimurium in the standard Ames Salmonella/microsome in vitro mutagenicity assay. Compound T-3290CoC was also screened for recombinogenic activity with the yeast Saccharomyces cerevisiae D3 assay. Both assays were performed in the presence and absence of a rat-liver metabolic activation system. Compound T-3290CoC was found to be neither mutagenic nor recombinogenic when tested using these procedures.

CONTENTS

INTRODUCTION

SRI International examined 3M Company's Compound T-3290CoC for mutagenicity by *in vitro* microbiological assays with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium *Salmonella typhimurium* in the standard Ames *Salmonella*/microsome assay and with the yeast *Saccharomyces cerevisiae* D3. An Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system was included in the assay procedures to provide metabolic steps that the microorganisms either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with *S. typhimurium* has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic. The assay procedure with *S. cerevisiae* is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination. However, because the assay systems do not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response conclusively proves that a chemical is carcinogenic or noncarcinogenic to man.

MATERIALS

- Test Compound

  - Name: T-3290CoC
  - Date Received: 8 October 1982
  - Description: Yellow-amber liquid
  - Storage Conditions: Room temperature
  - Special Testing Conditions: None

- Indicator Organisms

  - Species: Salmonella typhimurium LT2
    Saccharomyces cerevisiae

  - Strains: TA1535, TA1537, TA1538, TA98, and TA100 for S. typhimurium; D3 for S. cerevisiae

- Metabolic Activation

  Aroclor 1254-induced rat-liver S-9; SRI Batch E-8;
  ~ 31 mg/ml protein.

- Solvent Used

  Sterile water

METHODS

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence (his$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The his$^+$ revertants are easily visible as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased, usually in a dose-related manner.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley. In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB). The rfa mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The uvrB mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically induced DNA lesions and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an

increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cell. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz-(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept frozen in nutrient broth supplemented with 10% sterile glycerol at -80°C in 1-ml samples containing about 10^9 cells. New frozen stock cultures are made every 3 months from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, the frozen 1-ml samples are allowed to thaw at room temperature before inoculation in 50 ml of glucose minimal liquid medium supplemented with an excess of biotin and histidine. The cultures are grown at 37°C, unshaken for 4 hours, then gently shaken (100 rpm) for 11 to 14 hours. All strains are genetically analyzed whenever experiments are performed.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amine type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens. Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use. In brief, adult male Sprague-Dawley rats (200 to 250 g) are given a single 500 mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyls). This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the

animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed with an equal volume of cold, sterile 0.15 M KCl minced with sterile surgical scissors in three volumes of 0.15 M KCl, (3 ml/g of wet organ), and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 × g, and the supernatant, referred to as the S-9 fraction, is quickly frozen on dry ice and stored at -80°C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Plate Incorporation Assay

Prior to testing, the test article is serially diluted from an initial stock. The dose levels are based on the results of a preliminary range-finding experiment. The article is usually tested over a minimum of six dose levels, the highest nontoxic dose level being 10 mg/plate unless solubility, mutagenicity, or toxicity dictates a lower upper limit. All assays are repeated at least once on a separate day.

The plate incorporation assay is performed in the following way. To a sterile 13 × 100 mm test tube placed in a 43°C heating block we add:

(1) 2.00 ml of 0.6% agar containing 0.6% NaCl, 0.05 mM biotin and 0.05 mM histidine
(2) 0.05 ml of indicator organisms (about 10^8 bacteria)
(3) 0.05 ml of a solution of the test article
(4) 0.50 ml of metabolic activation mixture (if appropriate).

This mixture is stirred gently and then poured on plates containing about 25 ml of minimal glucose agar. After the top agar has set, the plates are incubated for 48 hours at 37°C. The number of *his*$^+$ revertant colonies is counted using a BioTran II automated colony counter when possible. When accurate counts cannot be obtained (e.g., because of precipitate), the plates are counted manually using an electric probe colony counter.

Concurrent sterility, negative (solvent), and positive controls are run with every experiment. Sterility controls include plating out separately steps (3) and (4). For negative controls, we use steps (1), (2), (4), and 0.05 ml of the solvent used for the test article. For positive controls, we test each bacterial culture with the following mutagens using steps (1), (2), (3), and (4):

- Sodium azide for the base-pair substitution mutants TA1535 and TA100.
- 9-Aminoacridine for the frameshift mutant TA1537.
- 2-Nitrofluorene for the frameshift mutants TA1538 and TA98.
- 2-Anthramine for all tester strains, in the presence of metabolic activation.

Saccharomyces cerevisiae D3

The yeast *S. cerevisiae* D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various carcinogenic

or recombinogenic agents. The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates.

A stock culture of S. cerevisiae is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C with shaking.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged and the cells are resuspended at a concentration of 10^8 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.00 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of the test chemical
- 0.30 ml of buffer.

Several doses of the test chemical are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline, and 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 2.0% agar; five plates are spread with the 10^{-3} dilution and three plates are spread with the 10^{-5} dilution. The plates are incubated for 3 days at 30°C, followed by 1 day at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10 × magnification, and the number of mitotic recombinants (red colonies or red sectors) is recorded. The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the 10^{-5} dilution.

The number of mitotic recombinants is calculated per 10^5 survivors. A positive response in this assay is indicated by a dose-related increase of more than 3-fold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

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RESULTS AND DISCUSSION

3M Company's Compound T-3290CoC was screened for mutagenic activity with the standard Ames Salmonella/microsome in vitro mutagenicity assay using the five standard Ames strains of Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, and TA100. This compound was assayed on two separate days, 11 October and 18 October 1982, each time over a dose range of 10 to 5,000 µg/plate, with two plates per dose level, using sterile water as the solvent. All assays were performed both in the presence and in the absence of a rat-liver metabolic activation system. This compound foamed when vortexed; however, this did not appear to interfere with the testing. No dose-related increase in the number of histidine-independent revertants was observed in either of the two assays. Therefore, we conclude that Compound T-3290CoC is nonmutagenic when tested by these procedures. Data from these assays are presented in Tables 1 and 2.

Compound T-3290CoC was also assayed for recombinogenic activity using the yeast Saccharomyces cerevisiae D3 assay for mitotic recombination. This assay was performed on two separate days, 11 October and 18 October 1982. This compound was tested twice over the dose range of 0.05% to 5.0%, both with and without a rat-liver metabolic activation system. Compound T-3290CoC was found to be reproducibly nonrecombinogenic when tested by these procedures. No dose-related increase in the number of mitotic recombinants per 10^5 survivors was observed. Data from these assays are presented in Tables 3 and 4.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

Compound T-3290CoC

Experiment Date: 11 October 1982

Compound	Metabolic Activation	Compound Added per plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Controls												
Sterile water	-		21	20	4	11	10	12	19	24	176	154
	+		7	13	7	9	16	17	25	33	179	139
Positive Controls												
Sodium Azide	-	1 µg	528	509							507	464
9-Aminoacridine	-	50			173	166						
2-Nitrofluorene	-	5					605	595	285	283		
2-Anthramine	-	1					17	20	19	19	173	184
	+	1					111	116	129	101	392	441
	-	2.5	21	18	8	9						
	+	2.5	105	115	69	65						
Compound T-3290CoC	-	10	25	24	7	13	16	14	18	19	164	155
	-	50	17	11	9	6	17	10	18	19	170	165
	-	100	24	15	6	6	18	20	21	24	164	161
	-	500	25	14	7	8	11	13	17	23	159	177
	-	1000	17	21	8	5	10	11	18	18	171	164
	-	5000	18	20	6	5	11	12	14	21	153	139
	+	10	15	9	8	6	18	29	28	27	162	160
	+	50	8	9	9	6	19	26	31	28	144	171
	+	100	8	7	9	7	18	19	36	21	177	167
	+	500	7	9	5	7	17	14	39	39	131	170
	+	1000	7	9	5	3	18	21	33	26	176	164
	+	5000	7	6	2	10	16	18	24	21	166	151

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

Compound T-3290CoC

Experiment Date: 18 October 1982

Compound	Metabolic Activation	Compound Added per plate	Histidine Revertants per Plate									
			TA1535		TA1537		TA1538		TA98		TA100	
Negative Controls												
Sterile water	-		21	16	4	6	12	6	17	13	152	122
	+		6	5	5	6	13	18	24	20	128	137
Positive Controls												
Sodium Azide	-	1 µg	377	445							381	411
9-Aminoacridine	-	50			94	103						
2-Nitrofluorene	-	5					450	462	292	277		
2-Anthramine	-	1					10	12	22	14	122	116
	+	1					158	171	187	170	387	445
	-	2.5	16	15	4	6						
	+	2.5	100	127	61	47						
Compound T-3290CoC	-	10	20	19	6	4	13	16	24	13	111	125
	-	50	29	18	5	3	9	8	19	19	117	101
	-	100	18	16	4	8	8	17	14	18	110	137
	-	500	11	17	8	6	9	12	11	19	146	125
	-	1000	16	24	6	5	10	10	20	17	116	160
	-	5000	15	15	12	7	11	12	21	19	137	126
	+	10	10	13	11	9	24	20	31	21	130	124
	+	50	7	9	6	6	20	26	20	19	116	145
	+	100	8	7	6	8	20	16	29	30	101	134
	+	500	8	6	7	4	27	29	25	28	122	116
	+	1000	4	6	7	5	18	15	31	20	137	117
	+	5000	5	14	12	7	20	14	14	19	135	131

Table 3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

Compound T-3290CoC

Experiment Date: 11 October 1982

Compound	Metabolic Activation	Percent Concentration weight/volume	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Controls						
Sterile water	-		4.8	100	4	8.3
	+		5.3	100	6	11.3
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	5.2	100	877	1686
Sterigmatocystin	-	0.0005	4.9	100	5	10.2
	+	0.0005	5.7	100	399	700
Compound T-3290CoC	-	0.05	4.6	96	2	4.3
	-	0.1	4.4	92	2	4.5
	-	0.5	5.1	100	4	7.8
	-	1	4.9	100	4	8.2
	-	5	4.5	94	1	2.2
	+	0.05	4.8	91	3	6.3
	+	0.1	4.7	89	4	8.5
	+	0.5	4.7	89	2	4.3
	+	1	5.0	94	3	6
	+	5	4.0	75	6	15

Table 4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

Compound T-3290CoC

Experiment Date: 18 October 1982

Compound	Metabolic Activation	Percent Concentration weight/volume	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
Negative Controls						
Sterile water	-		4.0	100	3	7.5
	+		4.1	100	6	14.6
Positive Controls						
1,2,3,4-Diepoxybutane	-	0.025	4.6	100	946	2057
Sterigmatocystin	-	0.0005	4.3	100	3	7.0
	+	0.0005	4.3	100	414	963
Compound T-3290CoC	-	0.05	3.8	95	4	10.5
	-	0.1	3.6	90	6	16.7
	-	0.5	3.6	90	5	13.9
	-	1	3.5	88	5	14.3
	-	5	3.8	95	2	5.3
	+	0.05	4.4	100	4	9.1
	+	0.1	4.3	100	4	9.3
	+	0.5	4.2	100	2	4.8
	+	1	4.7	100	4	8.5
	+	5	3.6	88	3	8.3

T-3991

28 Dermal Percutaneous Absorption Study with FC-128 in Albino Rabbits

Experiment No.: 0979AB0629

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: October 25, 1979 to December 17, 1979

Conducted By:

K. D. O'Malley, BS — Date
Advanced Toxicologist
Study Director

Reviewed By:

K. L. Ebbens, BS — Date
Supervisor, Acute Toxicology

dc: M. T. Case
K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

A 28 day percutaneous absorption study with FC-128 was conducted from October 25, 1979 to December 17, 1979 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female albino rabbits ranging in body weight from 1.95 to 2.90 kg. The test article was administered by dermal application to ten male and ten female rabbits a dosage level of 2,000 mg/kg body weight for a 24 hour exposure period.[a] No mortalities or untoward behavorial reactions were noted during the 28 day study. Body weight losses were noted in three females at the end of the study. Necropsies were performed on all animals upon termination of the study with no visible lesions noted. Preliminary serum analysis (See Appendix V) indicates dermal absorption of FC-128 in albino rabbits, however, due to the limited number of samples analyzed by the sponsor, no concrete conclusion may be drawn.

Introduction

The objective of this study[b] was to determine the percutaneous absorption potential of FC-128 in male and female albino rabbits. The study, which was initiated at Riker Laboratories, Inc., St. Paul, Minnesota on October 25, 1979 and completed on December 17, 1979, was not conducted to support a government submission or marketing permit and is therefore not regulated by the Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

[a] A preliminary rangefinder study was conducted to determine the appropriate dosage level to be used in this study.

[b] Riker Toxicity Experiment No.: 0979AB0629, Test Method 699

Method

Young adult albino rabbits of the New Zealand breed[a] were used in this test. All animals were held under quarantine for several days prior to testi with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rabbits were housed individ ually in stainless steel, wire-bottomed cages and maintained on a standard laboratory ration[b] with food and water available ad libitum.

An initial rangefinding study was conducted using two male and two female rabbits for each dosage level. The trunk of each animal was clipped free of hair and the test article placed on the surface of the intact skin which covered approximately 40% total body surface area. After administration of the test article, a flexible plastic collar was fitted on each animal and the trunk wrapped with impervious plastic sheeting which will occlude the test article. The animals were returned to their cages for a 24 hour period after which time the test article was removed from the dermal surface of the animals. The animals were observed for pharmacotoxic reactions both during the exposure period (immediately post dose administration, one and two hours) and after removal of the test article (daily for 14 days following dose administration) with all reactions recorded (Table 3). Initial and final body weights were also recorded (Table 1).

The information derived from the initial rangefinder was used in determining the dosage level for the 28 day percutaneous study. Preparation of 10 male and 10 female animals for dosing and application of the test article were conducted in the same manner as the rangefinder study with the exception of the collection of blood samples from the orbital sinus plexus prior to application and again on days 1, 7, 14 and 28 after initiation of the study for serum which was frozen for sponsor analysis. After the 24 hour exposure

[a] Pel Freez, Inc., Rogers, AR
[b] Purina Rabbit Chow, Ralston Purina, St. Louis, MO

period the test article was removed from the dermal surface of the animals and the animals returned to their cages for the following 28 days. Initial, 7, 14 and 28 day body weights were recorded (Table 2) as were any pharmacotoxic signs noted during the 28 day observation period (Table 4). A gross necropsy was conducted on all animals sacrificed on day 28 and all findings recorded (Table 2). The protocol, principal personnel involved in the study, composition characteristics, and Quality Assurance statement are contained in Appendices I - IV.

TABLE 1

ACUTE DERMAL RANGEFINDER TOXICITY STUDY - ALBINO RABBITS

with FC-128

Mortality and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number 0	Test Day Number 14	Number Dead / Number Tested	Percent Dead
5000	M	9B2582	2.16	1.18	0/4	0
	M	9B2585	2.34	1.96		
	F	9B2629	2.07	1.95		
	F	9B2632	2.00	1.94		
2000	M	9B2598	2.30	2.25	1/4	25
	M	9B2601	2.26	(3 days)		
	F	9B2648	1.98	1.97		
	F	9B2627	2.34	2.13		
1000	M	9B2604	2.32	2.19	0/4	0
	M	9B2607	2.41	2.57		
	F	9B2630	2.03	1.87		
	F	9B2633	2.20	1.99		

[a] Test article was dosed as a suspension in water

TABLE 2

ACUTE PERCUTANEOUS ABSORPTION TOXICITY STUDY - ALBINO RABBITS

with FC-128

Mortality and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number 0	7	14	28	Number Dead / Number Tested	Percent Dead
2000	M	9B3001	2.52	2.35	2.57	2.88	0/10	0
	M	9B3007	2.35	2.43	2.66	2.96		
	M	9B3013	2.59	2.26	2.52	2.84		
	M	9B3003	2.50	2.04	2.39	2.77		
	M	9B3009	2.09	2.15	2.26	2.51		
	M	9B3015	2.31	2.37	2.59	2.87		
	M	9B3005	2.13	1.85	2.10	2.46		
	M	9B3011	2.04	1.71	1.87	2.50		
	M	9B3074	2.07	1.98	2.29	2.49		
	M	9B3078	2.06	2.18	2.43	2.75		
2000	F	9B2969	1.95	1.79	2.05	2.48	0/10	0
	F	9B2975	2.13	2.18	2.40	2.67		
	F	9B2981	2.21	2.24	2.54	2.82		
	F	9B2987	2.34	2.33	2.44	2.70		
	F	9B2971	2.41	1.87	2.05	2.37		
	F	9B2977	2.13	1.91	2.25	2.35		
	F	9B2983	2.90	1.94	2.13	2.37		
	F	9B2989	2.21	1.97	2.12	2.36		
	F	9B2973	2.19	2.14	2.37	2.50		
	F	9B2979	2.38	1.73	1.57	1.79		

[a] Test article was dosed as a suspension in water

Necropsy

Necropsies performed upon termination of the study revealed no visible lesions

TABLE 3

ACUTE DERMAL RANGEFINDER TOXICITY STUDY - ALBINO RABBITS

with FC-128

Summary of Reactions

Dose (mg/kg)	Sex	Reaction	Number Affected / Number Dosed	Time of Onset [a] Following Dose Administration	Cessation of Reaction [b] Following Dose Administration	Time of Death Following Dose
5,000	M	Hypoactivity	1/2	Day 9	Day 14	
		Lethargy	1/2	Day 14	Until termination	
	F	No significant reaction		---	---	
2,000	M	No significant reaction		---	---	
	F	No significant reaction		---	---	
1,000	M	No significant reaction		---	---	
	F	No significant reaction		---	---	

[a] Time when first animal in the dose group exhibited the reaction
[b] Time when no animal in the dose group exhibited the reaction

TABLE 4

ACUTE PERCUTANEOUS ABSORPTION TOXICITY STUDY - ALBINO RABBITS

with FC-128

Summary of Reactions

Dose (mg/kg)	Sex	Reaction	Number Affected / Number Dosed	Time of Onset Following Dose Administration	Cessation of Reaction Following Dose Administration	Time of Death Following Dose
2,000	M	No significant reaction		---	---	---
	F	No significant reaction		---	---	---

Riker Experiment No.: 0479ABC629

APPENDIX I
PROTOCOL

8.

TEST: Single Dose 28 Day Percutaneous Absorption Study
SPONSOR: 3M Commercial Chemical Division
CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota
TEST ARTICLE: FC-128
CONTROL ARTICLE: N/A
PROPOSED STARTING/COMPLETION DATE OF STUDY: 11-79 / 1-80
TEST SYSTEM AND SOURCE: New Zealand White Albino Rabbits
Pel Freez, Inc., Rogers, Arkansas
Sex: M+F
Number: 10+10
Weight Range: 2-3 kg

OBJECTIVE: The objective of this study will be to determine the percutaneous absorption potential of the test article in albino rabbits. Rabbits were selected as the test system for their historical use in dermal absorption studies, ease of handling and general availability.

METHOD: The animals, selected from a larger colony by health and body weight, will be randomly housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The trunk of each animal will be clipped free of hair and the test article applied as a single dosage of 2,000 mg/kg to intact skin covering approximately 10% total body surface area. A flexible plastic collar[b] will be fitted on each animal and the trunk wrapped with impervious plastic sheeting, which will occlude the test article. The animals will then be returned to their cages for a 24 hour exposure period after which the test article will be removed. Prior to the application, blood samples will be collected from the orbital sinus plexus and again on days 1, 7, 14, and 28 after initiation of the study for serum which will be frozen for sponsor analysis. A gross necropsy will be conducted on all animals which may die during the conduct of the study as well as all animals sacrificed on day 28. All gross findings will be recorded and tissue samples of liver, spleen, brain kidney and bone marrow (sternum) will be fixed in 10% buffered formalin for possible future microscopic examination. Initial, 7, 14, and 28 day body weights will be recorded as well as any pharmacotoxic signs noted during the conduct of the study. All raw data, other than the blood analysis data which will be the responsibility of the sponsor, and the final report will be stored in the Riker Laboratory's Archives, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina, St. Louis, Missouri
[b] The collar will be worn for the duration of the study to reduce oral ingestion of residual test article.

W.C. McCormick /mm 11/7/79
Sponsor Date

Study Director 11/7/79
Date

Riker Experiment No.: [illegible]

APPENDIX I (Continued)
PROTOCOL

9.

TEST: Acute Dermal Toxicity Rangefinding Study
SPONSOR: 3M Commercial Chemical Divisi
CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesc
TEST ARTICLE: EC 128
CONTROL ARTICLE: N/A
PROPOSED STARTING/COMPLETION DATE OF STUDY: 11/29 - 12/80
TEST SYSTEM AND SOURCE: New Zealand White Albino Rabbits Sex: ♂/♀
Pel-Freez, Inc., Rogers, Arkansas Number: 2/2
Body Weight Range: 2-3kg

OBJECTIVE: The objective of this study will be to approximate the acute dermal toxicit of the test article in albino rabbits. Rabbits were selected as the test system for their sensitivity of response, historical data, ease of handling and general availability.

METHOD: The animals, selected from a larger colony by health and weight, will be randomly housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The trunk of each animal will be clipped free of hair and the test article placed on the surface of the intact skin at singl dosages of 5,000, 2,000, 1,000 mg/kg, however, if these dosage levels d not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels Any additional dosage levels will be documented and filed with this protoco The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, a flexible plastic collar[b] will be fitted on each animal and the trunk wrapped with impervious plastic sheeting which will occlude the test article. The anim will be returned to their cages for a 24 hour exposure period after which time the test article will be removed from the dermal surface of the animal The animals will be observed for pharmacotoxic reactions both during the ex posure period (immediately post dose administration, one and two hours) and after removal of the test article (daily for 14 days following dose adminis tration) with all reactions being recorded. Initial and final body weights will also be recorded. The acute median lethal dose (LD50) of the test article will be approximated. All raw data and the final report will be st in the Riker Laboratories Archives, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina, St. Louis, Missouri

[b] The collar will be worn for the duration of the study to reduce oral ingestion of residual test article.

N/A
Sponsor Date

[signature]
Study Director 11/10/79

Riker Experiment No.: 079HBX029

APPENDIX I (Concluded)
Amendment to Protocol

1. The study director and corresponding number will be changed to K.D O'Malley (09)

K D O'Malley / 11/10/79
Study Director Date

2. The 1-30 minute, 60 minute and 120 minute evaluations were not conducted due to the nature of the study.

K D O'Malley 10/25/79
Study Director Date

3. The weight range is extended to 19-30 kg in order to initiate the study in a timely manner.

K D O'Malley 11/19/79
Study Director Date

4. Due to delays in expensive serum analyses and report processing, the completion date is extended to 3/81

K D O'Malley 7/16/80
Study Director Date

5. _____
Study Director Date

6. _____
Study Director Date

7. _____
Study Director Date

8. _____
Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
K. L. Ebbens, BS	Supervisor, Acute Toxicology
K. D. O'Malley, BS	Advanced Toxicoligist Study Director
Dr. V. Pothapragada	Commercial Chemicals Chemist
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore a statement signed and prepared by the Quality Assurance group is not applicable. This study was, however, audited by the Quality Assurance group.

In addition to the data audit, different significant phases for studies underway in the Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Laboratory Quality Assurance on a three month schedule.

Internal Correspondence

cc: D. R. Ricker - 3M-1

FC: F. D. Griffith - 220-2E
F. A. Ubel - 220-2E

APPENDIX V

To: K. L. EBBENS - RIKER SAFETY EVALUATION LAB - 203-1

From: W. C. McCORMICK - MEDICAL DEPT. - TOXICOLOGY SERVICES - 220-2E

Subject: SKIN ABSORPTION STUDIES ON FC-143, FC-95, FC-99, FC-134, FC-135, FC-128, FC-129 and FC-98

Date: JUNE 27, 1980

3M

Please consider this an authorization for your laboratory to release the dermal toxicity/skin absorption studies conducted on the above mentioned compounds.

It is understood that the studies are being issued in an incomplete form insofar as the fluorochemical analysis of the serum samples have not been completed and will not be included in the report. Preliminary serum sample analysis indicates absorption of the compounds. The serum data analysis are not sufficient enough to draw any concrete conclusions concerning comparitive toxicity. However, the animal data you have generated addresses this matter in a broader context. It is not certain when the remaining samples will be analyzed and their completion should not hold up your report any longer.

Thank you for your patience in this matter.

W. C. McCormick

WCM:klh

Internal Correspondence

cc: [illegible]
R. A. Prokop - 236-2B
L. D. Winter - 236-2B

APPENDIX V (Concluded)

COMMERCIAL CHEMICALS DIVISION
ANALYTICAL LAB REPORT #146

To W. C. MCCORMICK - 220-2E-02

From V. POTHAPRAGADA AND V. BUNNELLE - 236-3A

Subject RIKER SKIN ABSORPTION STUDY

Date June 9, 1980

Reference: Commercial Chemicals Division Analytical Request #15669

For lack of time, only a selected set of serum samples was analyzed.

	TOTAL F, ppm			
	Females		Males	
Compound	Day 1	Day 28	Day 1	Day 28
FC-129	26.1	69.6	11.4	23.3
FC-134	0.2	18.1	18.8	23.9
FC-128	4.4	16.5	1.6	10.5
FC-98	226.4	93.1	271.9	94.3
FC-135	6.9	20.8	2.3	7.6
FC-95	0.9	128.0	10.3	130.2
FC-99	42.5	111.5	129.1	73.5
	53.1	119.8	72.7	66.6

	Females			Males		
	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
FC-143	10.1	12.1	3.5	5.4	6.8	4.6

Method of Analysis: Oxygen Bomb/GC Technique (Jon Bellido and D. F. Hagen, Anal. Biochem; 87, 545, 1978).

V. A. Bunnelle
V. A. Bunnelle

V. Pothapragada
V. Pothapragada

Read and Reviewed
by L. D. Winter

VAB/hc

T-398

28 Day Percutaneous Absorption Study with FC-129 in Albino Rabbits

Experiment No.: 0979AB0627

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: October 24, 1979 to December 18, 1979

Conducted By:

K. D. O'Malley, BS — Date
Advanced Toxicologist
Study Director

Reviewed By:

K. L. Ebbens, BS — Date
Supervisor, Acute Toxicology

dc: M. T. Case
K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

A 28 day percutaneous absorption study with FC-129 was conducted from October 24, 1979 to December 18, 1979 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female albino rabbits ranging in body weight from 1.75 to 2.42 kg. The test article was administered by dermal application to ten male and ten female rabbits at a dosage level of 5,000 mg/kg body weight for a 24 hour exposure period[a]. Six mortalities were noted which occurred between days two and three. The untoward behavioral reactions which were noted during the 28 day study consisted of lethargy, hypoactivity, prostration and blood was noted the urine. Onset of the reactions occurred from day 1 to day 6 and all reactions subsided by day 7 or death precluded recovery. Body weight losses were noted in two of the animals which survived the observation period. Necropsies of animals which died acutely, generally revealed pale, mottled livers and blood in the urine with one animal exhibiting a dark green spot on the brain. Necropsies were also performed on animals which survived the study period and revealed no visible lesions, with the exception of one animal which had an atrophic spleen. Preliminary serum analysis (see Appendix V) indicates dermal absorption of FC-129 in albino rabbits, however, due to the limited number of samples analyzed by the sponsor, no concrete conclusion may be drawn.

Introduction

The objective of this study[b] was to determine the percutaneous absorption potential of FC-129 in male and female albino rabbits. The study, which was initiated at Riker Laboratories, Inc., St. Paul, Minnesota on October 24, 1979

a A preliminary rangefinder study was conducted to determine the appropriate dosage level to be used in this study.

b Riker Toxicity Experiment No.: 0979AB0627, Test Method 699

and completed on December 18, 1979, was not conducted to support a government submission or marketing permit and is therefore not regulated by the Good Laboratory Practice Regulation of 1978. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method

Young adult albino rabbits of the New Zealand breed[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rabbits were housed individually in stainless steel, wire-bottomed cages and maintained on a standard laboratory ration[b] with food and water available ad libitum.

An initial rangefinding study was conducted using two male and two female rabbits for each dosage level. The trunk of each animal was clipped free of hair and the test article placed on the surface of the intact skin which covered approximately 40% total body surface area. After administration of the test article, a flexible plastic collar was fitted on each animal and the trunk wrapped with impervious plastic sheeting which will occlude the test article. The animals were returned to their cages for a 24 hour period after which time the test article was removed from the dermal surface of the animals. The animals were observed for pharmacotoxic reactions both during the exposure period (immediately post dose administration, one and two hours) and after removal of the test article (daily for 14 days following dose administration) with all reactions recorded (Table 3). Initial and final body weights were also recorded (Table 1).

The information derived from the initial rangefinder was used in determining the dosage level for the 28 day percutaneous study. Preparation of 10 male and 10 female animals for dosing and application of the test article were conducted in the same manner as the rangefinder study with the exception of the collection of blood samples from the orbital sinus plexus prior to application and again on days 1, 7, 14 and 28 after initiation of the study for serum which was frozen for sponsor analysis. After the 24 hour exposure

[a] Pel Freez, Inc., Rogers, AR
[b] Purina Rabbit Chow, Ralston Purina, St. Louis, MO

period the test article was removed from the dermal surface of the animals and the animals returned to their cages for the following 28 days. Initial, 7, 14 and 28 day body weights were recorded (Table 2) as were any pharmacotoxic signs noted during the 28 day observation period (Table 4). A gross necropsy was conducted on all animals sacrificed on day 28 and all findings recorded (Table 2). The protocol, principal personnel involved in the study, composition characteristics, and Quality Assurance statement are contained in Appendices I - IV.

TABLE 1

ACUTE DERMAL RANGEFINDER TOXICITY STUDY - ALBINO RABBITS

with FC-129

Mortality and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number 0	14	Number Dead / Number Tested	Percent Dead
5000	M	9B2593	2.22	1.29	0/4	0
	M	9B2591	2.19	1.44		
	F	9B2689	2.07	1.41		
	F	9B2646	2.26	2.03		
2000	M	9B2594	2.10	1.76	0/4	0
	M	9B2597	2.09	2.00		
	F	9B2684	2.17	2.14		
	F	9B2687	2.02	1.87		
1000	M	9B2574	2.20	2.31	0/4	0
	M	9B2577	2.29	2.37		
	F	9B2690	2.37	2.24		
	F	9B2693	2.16	2.18		

[a] Test article was dosed undiluted

TABLE 2

ACUTE PERCUTANEOUS ABSORPTION TOXICITY STUDY - ALBINO RABBITS

with FC-129

Mortality and Body Weight Data

Dose[a] (mg/kg)	Sex	Animal Number	Individual Body Weights (kg) Test Day Number 0	7	14	28	Number Dead / Number Tested	Percent Dead
5000	M	9B3056	2.20	1.82	1.78	2.18	1/10	10
	M	9B3062	2.40	1.97	2.11	2.41		
	M	9B3057	2.42	2.14	2.43	2.75		
	M	9B3063	2.10	(2 Days)	----	----		
	M	9B3072	1.75	1.91	2.12	2.44		
	M	9B3077	2.04	2.04	2.05	2.30		
	M	9B3032	2.04	1.59	1.73	2.18		
	M	9B3038	2.38	2.15	2.37	2.45		
	M	9B3033	2.36	2.23	2.23	2.60		
	M	9B3039	2.28	1.98	2.25	2.42		
5000	F	9B2985	2.23	(3 Days)	----	----	5/10	50
	F	9B2991	2.17	(3 Days)	----	----		
	F	9B2936	2.23	(2 Days)	----	----		
	F	9B2942	2.00	1.61	1.56	1.84		
	F	9B2960	2.37	(2 Days)	----	----		
	F	9B2966	2.07	1.70	2.05	2.43		
	F	9B2955	1.87	1.89	2.15	2.44		
	F	9B2967	2.04	1.81	2.05	2.33		
	F	9B2937	1.98	1.72	2.04	2.32		
	F	9B2943	2.05	(2 Days)	----	----		

[a] Test article was dosed undiluted

Necropsy

Necropsies performed on animals which died acutely, generally revealed pale mottled liver and blood in urine, with one animal having a dark green spot on the brain. Animals which were sacrificed upon termination of the study revealed no visible lesions, with the exception of one animal which had an atrophic spleen.

TABLE 3

ACUTE DERMAL RANGEFINDER TOXICITY STUDY - ALBINO RABBITS

with FC-129

Summary of Reactions

Dose (mg/kg)	Sex	Reaction	Number Affected / Number Dosed	Time of Onset [a] Following Dose Administration	Cessation of Reaction [b] Following Dose Administration	Time of Death Following Dose
5000	M	Hypoactivity	2/2	Day 9	until termination	---
	F	No significant reactions		---	---	---
2000	M	No significant reactions		---	---	---
	F	No significant reactions		---	---	---
1000	M	No significant reactions		---	---	---
	F	No significant reactions		---	---	---

[a] Time when first animal in the dose group exhibited the reaction

[b] Time when no animal in the dose group exhibited the reaction

TABLE 4

ACUTE PERCUTANEOUS ABSORPTION TOXICITY STUDY - ALBINO RABBITS

with FC-129

Summary of Reactions

Dose (mg/kg)	Sex	Reaction	Number Affected / Number Dosed	Time of Onset Following Dose Administration	Cessation of Reaction Following Dose Administration	Time of Death Following Dose
5,000	M	Hypoactivity	1/10	Day 6	Day 7	
		Lethargy	2/10	Day 5	Day 6	
		Prostration	1/10	Day 1	Until death	Day 2
		Blood in urine	1/10	Day 1	Until death	Day 2
5,000	F	Hypoactivity	4/10	Day 1	Day 3	
		Prostration	1/10	Day 1	Until death	Day 2
		Blood in urine	2/10	Day 1	Until death	Day 2

[a]/[b] Time when first animal in the dose group exhibited the reaction
[a]/[b] Time when no animal in the dose group exhibited the reaction

Riker Experiment No.: 04-1980.4

APPENDIX I
PROTOCOL

9.

TEST: Single Dose 28 Day Percutaneous Absorption Study
SPONSOR: 3M Commercial Chemical
CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota Division
TEST ARTICLE: FC-129 Lot 510
CONTROL ARTICLE: N/A
PROPOSED STARTING/COMPLETION DATE OF STUDY: 11-79 / 1-80
TEST SYSTEM AND SOURCE: New Zealand White Albino Rabbits
Pel Freez, Inc., Rogers, Arkansas
Sex: M+F
Number: 10/10
Weight Range: 2-3 kg

OBJECTIVE: The objective of this study will be to determine the percutaneous absorption potential of the test article in albino rabbits. Rabbits were selected as the test system for their historical use in dermal absorption studies, ease of handling and general availability.

METHOD: The animals, selected from a larger colony by health and body weight, will be randomly housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The trunk of each animal will be clipped free of hair and the test article applied as a single dosage of 5,000 mg/kg to intact skin covering approximately 10% total body surface area. A flexible plastic collar[b] will be fitted on each animal and the trunk wrapped with impervious plastic sheeting, which will occlude the test article. The animals will then be returned to their cages for a 24 hour exposure period after which the test article will be removed. Prior to the application, blood samples will be collected from the orbital sinus plexus and again on days 1, 7, 14, and 28 after initiation of the study for serum which will be frozen for sponsor analysis. A gross necropsy will be conducted on all animals which may die during the conduct of the study as well as all animals sacrificed on day 28. All gross findings will be recorded and tissue samples of liver, spleen, brain, kidney and bone marrow (sternum) will be fixed in 10% buffered formalin for possible future microscopic examination. Initial, 7, 14, and 28 day body weights will be recorded as well as any pharmacotoxic signs noted during the conduct of the study. All raw data, other than the blood analysis data which will be the responsibility of the sponsor, and the final report will be stored in the Riker Laboratory's Archives, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina, St. Louis, Missouri
[b] The collar will be worn for the duration of the study to reduce oral ingestion of residual test article.

W. C. McCormick /KPO 11/7/79
Sponsor Date

K D Smalley 11/7/79
Study Director Date

Riker Experiment No.: 24-1911456

APPENDIX I (Continued)

PROTOCOL

1/ 10.

TEST: Acute Dermal Toxicity Rangefinding Study

SPONSOR: 3M Commercial Chemical

CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesot Divisi

TEST ARTICLE: FC-129

CONTROL ARTICLE: N/A

PROPOSED STARTING/COMPLETION DATE OF STUDY: 11/29 - 1/80

TEST SYSTEM AND SOURCE: New Zealand White Albino Rabbits Sex: ♂/♀
Pel-Freez, Inc., Rogers, Arkansas Number: 2/2
Body Weight Range: 2.3 kg

OBJECTIVE: The objective of this study will be to approximate the acute dermal toxicity of the test article in albino rabbits. Rabbits were selected as the test system for their sensitivity of response, historical data, ease of handling and general availability.

METHOD: The animals, selected from a larger colony by health and weight, will be randomly housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered ad libitum. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. The trunk of each animal will be clipped free of hair and the test article placed on the surface of the intact skin at single dosages of 5,000, 2,000, 1,000 mg/kg, however, if these dosage levels do not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, a flexible plastic collar[b] will be fitted on each animal and the trunk wrapped with impervious plastic sheeting which will occlude the test article. The animal will be returned to their cages for a 24 hour exposure period after which time the test article will be removed from the dermal surface of the animals. The animals will be observed for pharmacotoxic reactions both during the exposure period (immediately post dose administration, one and two hours) and after removal of the test article (daily for 14 days following dose administration) with all reactions being recorded. Initial and final body weights will also be recorded. The acute median lethal dose (LD50) of the test article will be approximated. All raw data and the final report will be stor in the Riker Laboratories Archives, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina, St. Louis, Missouri

[b] The collar will be worn for the duration of the study to reduce oral ingestion of residual test article.

N/A
Sponsor Date

KD Dalley 11/29/79
Study Director Dat

APPENDIX I (Concluded)
Amendment to Protocol

Riker Experiment No.: 0979 AB0627

1. The 1-5 minute, 60 and 120 minute observations will be omitted due to the nature of the nasal findings.

K D Smalley — **Study Director** — 10/24/79 — **Date**

2. The study director number will be changed from 04 (K.L. Fabian) to 09 (K.D. Smalley)

K D Smalley — **Study Director** — 10/24/79 — **Date**

3. The weight range is extended to 1.7 - 30 kg in order to initiate the study

K D Smalley — **Study Director** — 11/20/79 — **Date**

4. Due to delays in sponsor serum analyses and report processing, the completion date is extended to 3/81

K D Smalley — **Study Director** — 7/16/80 — **Date**

5. _____

Study Director — **Date**

6. _____

Study Director — **Date**

7. _____

Study Director — **Date**

8. _____

Study Director — **Date**

11.

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
K. L. Ebbens, BS	Supervisor, Acute Toxicology
K. D. O'Malley, BS	Advanced Toxicologist
Dr. V. Pothapragada	Study Director
	Commercial Chemicals Chemist
G. C. Pecore	Supervisor
	Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore a statement signed and prepared by the Quality Assurance group is not applicable. This study was, however, audited by the Quality Assurance group.

In addition to the data audit, different significant phases for studies underway in the Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Laboratory Quality Assurance on a three month schedule.

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6051 and T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-133

Study Number: AMDT-013195.1
Test Substance: FC-129 (T-6051 and T-6054)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/22/95
Completion Date

1.0 SUMMARY

Samples of liver from rabbits administered FC-129 (T-6054) or FC-129 treated fabric (T-6051) were analyzed at 28 days post dermal administration for total organic fluorine and perfluorooctanesulfonate. The results show that for the highest liquid formulation dose group (12.8 mg/kg) there is on the average about 0.2% of the dose in whole liver at 28 days.

Thus, dermal administration of FC-129 at higher levels results in some dermal absorption.

2.0 INTRODUCTION

Two studies were performed on FC-129. A pharmacokinetic study (HWI#6329-138) and this dermal absorption study (HWI #6329-133). The pharmacokinetic study showed that perfluorooctanesulfonate is a useful marker to assess the dermal absorption of FC-129. Liver, serum, and other tissues were available for analysis by combustion for total organic fluorine and electrospray mass spectrometry for analysis of specific molecules such as perfluorooctanesulfonate. By obtaining and then analyzing data from rabbits at 28 days post dermal dose, information for the assessment of the extent of dermal absorption of FC-129 is provided in this study.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company.

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20±10^0 C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-133), were available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests could be performed. The screening for fluoride in liver via combustion was the most likely analysis to present definitive data for absorption. Other available tests were electrospray mass spectroscopy and gas chromatography/mass spectrometry for metabolites. Liver samples were analyzed by both combustion and electrospray. Data were then analyzed to assess the extent of dermal absorption.

5.0 EXPERIMENTAL - METHODS

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

6.0 DATA ANALYSIS

The data are attached. The level of total organic fluorine in whole liver for the control, 0.128, 1.28, and fabric doses are below the practical quantitation limit for this method. Just using meter readings and extrapolating from the standard curve, the values are on the order of 16 ug/whole liver. The 12.8 mg/kg dermal dose however, results in detectable amounts of organic fluoride in whole liver at 28 days

post dose. The values range from below the practical quantitation limit for one of the 6 rabbits (F52895) to 75 ug/whole liver for rabbit F52889. The average is 45 ug/whole liver with a standard deviation of 21 ug.

Electrospray mass spectrometry data are in agreement with the combustion data; there is very little perfluorooctanesulfonate in the groups other than the 12.8 mg/kg group. Small detectable amounts are observed in the 1.28 mg/kg and fabric groups; however, these are estimated to be on the order of 17 ug/whole liver or less. For the 12.8 mg/kg dose group, perfluorooctanesulfonate is detected in all rabbit liver samples at 28 days post dermal dose. The amounts are estimated to range from 25 to 85 ug/whole liver (mean of 48 ug/whole liver). The rabbit that showed 75 ug/whole liver of total organic fluorine for combustion analysis (F52889), had 56 ug/whole liver perfluorooctanesulfonate.

From the pharmacokinetic study on FC-129 (HWI#6329-138), it is known that a good portion of the intravenous dose will be biotransformed to perfluorooctanesulfonate. It is known that the half-life of perfluorooctanesulfonate in rabbits is >1 month. Thus, if FC-129 is dermally absorbed a portion of it will appear in liver at 28 days as perfluorooctanesulfonate.

Fifty ug perfluorooctanesulfonate/whole liver is 0.2% of the dose for the 12.8 mg/kg rabbits assuming a body weight of 2 kg and expressing the dose in potassium perfluorooctanesulfonate equivalents (FC-95). After an intravenous dose of 12.8 mg/kg a rabbit had 1.05% of the dose in liver at 48 hours. For comparison, 1.05% with a biological half-life of 30 days would be approximately 0.5% of the dose at day 28. Thus, estimated levels after an intravenous dose of 12.8 mg/kg would be 0.5% of the dose in whole liver and estimated levels after dermal administration of 12.8 mg/kg would be 0.2% of the dose in whole liver if the levels are compared at 28 days.

Other data was collected using Skalar segmented flow analyzer with ion selective electrode (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances that May Have Affected the Quality of the Data: The problem with this analysis is that the extent of biotransformation of the rest of the fluorinated compounds in the liver at 48 hours in the pharmacokinetic study (HWI#6329-138) to perfluorooctanesulfonate is not known. There could be considerable biotransformation of the several percent of dose that fluorinated molecules other than perfluorooctanesulfonate represent. However, the 1.05% of the dose observed at 48 hours still indicates that perfluorooctanesulfonate is a sensitive marker to assess biotransformation of this compound. At 28 days, the value could

be somewhat higher than the above estimate of 0.5% of the dose due to delayed metabolism. If this were true, the value for dermal absorption has by definition (since it is measured at 28 days) a built in compensation for this delay and the value of 0.2% of the dose after dermal administration is being compared with a percentage of dose from the intravenous dose that is too low.

7.0 CONCLUSION

There is evidence of dermal absorption in rabbits of FC-129 after dermal administration of a 12.8 mg/kg dose.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-133: "Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-013195.1

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-013195.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.1.1 Protocol and Final Report: HWI#6329-133: "Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6054 and T-6051 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 16, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-133

Phone 608-241-4471
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON, WI 53704
Fax 608-241-7227

HWI 6329-133

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/08/94	12/09/94	Protocol Review	12/09/94	01/10/95
12/28/94	12/28/94	Dose Administration	12/28/94	01/10/95
01/09/95	01/09/95	Protocol Amendment	01/09/95	02/10/95
01/30/95	01/30/95	Protocol Amendment	01/30/95	02/10/95
03/17/95	03/21/95	Data/Report Review	03/21/95	04/10/95
03/17/95	03/21/95	Data Review	03/21/95	04/10/95
06/14/95	06/15/95	Report Rereview	06/15/95	07/10/95
06/16/95	06/16/95	Report Rereview	06/16/95	07/10/95

Stacy Thuck for
Cecilia M. Danner
Representative, Quality Assurance Unit

6-16-95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity
Study of T-6054 and T-6051 in Rabbits

Test Materials	1. T-6054 2. T-6051
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Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 13, 1994
Experimental (In-life) Start Date	December 28, 1994
In-life End Date	January 25, 1995
Experimental Termination Date	June 16, 1995
Study Completion Date	June 16, 1995

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SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6054 and T-6051 when applied to the skin of rabbits.

The study was conducted using three male and three female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group.

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Sterile water	0[a]	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	[b]	3	3

a Administered at a dose volume of 2.0 mL/kg.
b Administered as a 10-cm x 10-cm section of test material (fabric).

The back of each rabbit was clipped free of hair and a single dose of the respective material at the indicated dose level was administered to the skin of the rabbits. The treatment sites remained intact. The area of application was covered with a gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for a 24-hour exposure period.

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. Body weights were determined on Day -9 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before in-life initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6054 and T-6051 did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared clinically normal throughout the study with the exception of one female animal treated with T-6054 at 1.28 mg/kg that exhibited weakened hind limbs the last 22 days of study. This animal was also noted as having small feces on Day 8. These findings are considered to be due to an injury incurred during the sample collection procedures and are not considered to be test material-related. The control material and test material T-6051 did not produce any dermal irritation. No dermal irritation was observed as a result of T-6054 at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced very slight dermal irritation in five animals at the 12.8 mg/kg dose level.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of test materials when applied to the skin of rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test material mixtures prepared for the Groups 2, 3, and 4 animals for concentration, homogeneity/solubility, and stability was not conducted and the original test material usage log can not be located although a copy is retained in the study file. All procedures used in this study are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test materials were identified and described as follows:

Identification	Physical Description
T-6054	Amber liquid
T-6051	White plastic sheets

The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixtures prepared for the Groups 2, 3, and 4 animals for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test materials were stored at room temperature. The control material was stored refrigerated. A reserve sample of each test and control material was

taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any unused test material was returned to the Sponsor after completion of all in-life phase according to HWI SOP. Any remaining control material is retained for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Kalamazoo, MI, on December 14, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -9. The

weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. One female animal (No. F52890) was replaced in the study prior to treatment due to poor health. This animal was replaced with another female (No. F52877).

Study Design

Animals weighing from 2,157 to 2,508 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Sterile water	0[a]	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	[b]	3	3

a Administered at a dose volume of 2.0 mL/kg.
b Administered as a 10-cm x 10-cm section of test material (fabric).

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The test sites (intact skin) were inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped on Days 8 and 29 to aid in visualizing the application sites.

Dose Administration

All animals received a single administration of the respective test or control material. The day of treatment was designated as Day 1.

Group 1. An individual dose (2.0 mL/kg) was calculated and measured based on each animal's body weight on the day of treatment. The control material (sterile water for injection) was applied evenly to the test site at a rate of approximately 0.05 mL/cm^2.

Groups 2, 3, and 4. For the Groups 2, 3, and 4 animals (0.128, 1.28, 12.8 mg/kg, respectively), the test material (T-6054) was mixed with sterile water for injection to a concentration of 99, 990, and 9,920 mg/mL, respectively, and applied at a dose volume of 0.01 mL/kg. The mixtures were stored at room temperature until administered. An individual dose of the respective test material mixture was calculated for each animal based on its body weight on the day of treatment. For all three groups, the area of exposure was 4 cm^2 and the approximate rate of application was 0.006 mL/cm^2.

Group 5. The test material (T-6051) was applied to each animal's skin as a 10-cm x 10-cm section of material that was moistened with distilled water.

Each area of application was covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during the 24-hour exposure period.

Approximately 24 hours after test or control material application, the restraining collars and bandages were removed and any residual test material was removed with tap water and disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days.

Body weights were determined for randomization purposes on Day -9, before test material administration (Day 1), and at in-life termination (Day 29).

The initial dermal irritation reading was made before test or control material administration according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. The only exception to this was the Day 8 erythema score for one female animal (No. F52889) in Group 4 was inadvertently not recorded.

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of test and control material application were washed with lukewarm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106), along with their corresponding weights or volumes. The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the tissue samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1. All animals exhibited body weight gains from Day 1 to Day 29.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study with the exception of one female animal (No. F52900) treated with T-6054 at 1.28 mg/kg that exhibited weakened hind limbs during the last 22 days of study. This animal also had small feces on Day 8. These findings are considered to be due to an injury incurred during the sample collection procedures and are not considered to be test material-related.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material and test material T-6051 produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6054 at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced slight to moderate erythema reactions at Days 2 and 4 only in five animals at the 12.8 mg/kg dose level.

Pathology

Individual animal pathology comments are presented in Table 4. There were no lesions observed in any of the animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6054 and T-6051 were evaluated in male and female albino rabbits when administered as a single dermal application. Application of the these materials did not result in any test material-related effects on in-life clinical findings, body weight gain or macroscopic findings at necropsy. The control material and test material T-6051 did not produce any dermal irritation. No dermal irritation was observed with T-6054 applied at a dose level of 0.128 or 1.28 mg/kg. T-6054 produced very slight dermal irritation in five animals at the 12.8 mg/kg dose level.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-16-95
Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

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PATHOLOGY REPORT

There were six rabbits (three males and three females) each from five dose levels euthanized and necropsied at the termination of the study. The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, there were no visible lesions in any of the animals. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. After necropsy, the animals were discarded.

James Carter for Thomas Palmer

Thomas E. Palmer, PhD
Pathologist

6-16-95
Date

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Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -9	Day 1	Day 29	Animal Number	Randomization Day -9	Day 1	Day 29
Group 1 (Control) - Sterile Water for Injection (0 mg/kg)							
F52885	2,292	2,483	2,853	F52967	2,204	2,368	2,863
F52873	2,204	2,321	2,745	F52878	2,331	2,475	2,978
F52898	2,169	2,366	2,745	F52883	2,274	2,499	2,985
Mean	2,222	2,390	2,781		2,270	2,447	2,942
Group 2 - T-6054 (0.128 mg/kg)							
F52887	2,272	2,235	2,621	F52901	2,281	2,340	2,839
F52893	2,204	2,260	2,603	F52876	2,270	2,358	2,842
F52897	2,338	2,423	2,913	F52882	2,127	2,323	2,814
Mean	2,271	2,306	2,712		2,226	2,340	2,832
Group 3 - T-6054 (1.28 mg/kg)							
F52965	2,248	2,369	2,966	F52884	2,351	2,508	2,928
F52891	2,077	2,383	2,757	F52900	2,210	2,460	2,588
F52892	2,261	2,333	2,614	F52968	2,166	2,289	2,822
Mean	2,195	2,362	2,779		2,242	2,419	2,779
Group 4 - T-6054 (12.8 mg/kg)							
F52886	2,286	2,417	2,793	F52889	2,292	2,357	2,853
F52880	2,161	2,219	2,525	F52894	2,160	2,489	2,837
F52899	2,353	2,393	2,741	F52895	2,219	2,462	2,791
Mean	2,267	2,343	2,686		2,224	2,436	2,827

Table 1 (Continued)
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -9	Day 1	Day 29	Animal Number	Randomization Day -9	Day 1	Day 29
Group 5 - T-6051 (10-cm x 10-cm Section)							
F52879	2,278	2,398	2,923	F52877[a]	2,021	2,157	2,782
F52963	2,173	2,258	2,622	F52888	2,191	2,273	2,752
F52874	2,048	2,265	2,495	F52966	2,264	2,322	2,792
Mean	2,166	2,307	2,680		2,159	2,251	2,775

a Animal No. F52890 was originally selected by the randomization program for use in the study but was replaced with No. F52877 due to poor health.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 - 7	Day 8	Day 9 - 29
Group 1 (Control) - Sterile Water for Injection (0 mg/kg)						
Male	F52885	Appeared normal	✓	✓	✓	✓
	F52873	Appeared normal	✓	✓	✓	✓
	F52898	Appeared normal	✓	✓	✓	✓
Female	F52967	Appeared normal	✓	✓	✓	✓
	F52878	Appeared normal	✓	✓	✓	✓
	F52883	Appeared normal	✓	✓	✓	✓
Group 2 - T-6054 (0.128 mg/kg)						
Male	F52887	Appeared normal	✓	✓	✓	✓
	F52893	Appeared normal	✓	✓	✓	✓
	F52897	Appeared normal	✓	✓	✓	✓
Female	F52901	Appeared normal	✓	✓	✓	✓
	F52876	Appeared normal	✓	✓	✓	✓
	F52882	Appeared normal	✓	✓	✓	✓
Group 3 - T-6054 (1.28 mg/kg)						
Male	F52965	Appeared normal	✓	✓	✓	✓
	F52891	Appeared normal	✓	✓	✓	✓
	F52892	Appeared normal	✓	✓	✓	✓
Female	F52884	Appeared normal	✓	✓	✓	✓
	F52900	Appeared normal	✓	✓	-	-
		Weakened hind limbs	-	-	✓	✓
		Small feces	-	-	✓	-
	F52968	Appeared normal	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 - 7	Day 8	Day 9 - 29
		Group 4 - T-6054 (12.8 mg/kg)				
Male	F52886	Appeared normal	✓	✓	✓	✓
	F52880	Appeared normal	✓	✓	✓	✓
	F52899	Appeared normal	✓	✓	✓	✓
Female	F52889	Appeared normal	✓	✓	✓	✓
	F52894	Appeared normal	✓	✓	✓	✓
	F52895	Appeared normal	✓	✓	✓	✓
		Group 5 - T-6051 (10-cm x 10-cm Section)				
Male	F52879	Appeared normal	✓	✓	✓	✓
	F52963	Appeared normal	✓	✓	✓	✓
	F52874	Appeared normal	✓	✓	✓	✓
Female	F52877	Appeared normal	✓	✓	✓	✓
	F52888	Appeared normal	✓	✓	✓	✓
	F52966	Appeared normal	✓	✓	✓	✓

✓ Condition existed.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Sterile Water for Injection (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52885				Animal No. F52967			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52873				Animal No. F52878			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52898				Animal No. F52883			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

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Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6054 (0.128 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52887				Animal No. F52901			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52893				Animal No. F52876			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52897				Animal No. F52882			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6054 (1.28 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52965				Animal No. F52884			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52891				Animal No. F52900			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52892				Animal No. F52968			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

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Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6054 (12.8 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52886				Animal No. F52889			
Erythema	0	0	0	0	0	1	1	-
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52880				Animal No. F52894			
Erythema	0	1	1	0	0	1	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52899				Animal No. F52895			
Erythema	0	1	1	0	0	2	1	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

- Value not recorded.

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 5 - T-6051 (10-cm x 10-cm Section)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52879				Animal No. F52877			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52963				Animal No. F52888			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52874				Animal No. F52966			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
	Group 1 (Control) - Sterile Water for Injection (0 mg/kg)			
F52885	M	-	29	No visible lesions.
F52873	M	-	29	No visible lesions.
F52898	M	-	29	No visible lesions.
F52967	F	-	29	No visible lesions.
F52878	F	-	29	No visible lesions.
F52883	F	-	29	No visible lesions.
	Group 2 - T-6054 (0.128 mg/kg)			
F52887	M	-	29	No visible lesions.
F52893	M	-	29	No visible lesions.
F52897	M	-	29	No visible lesions.
F52901	F	-	29	No visible lesions.
F52876	F	-	29	No visible lesions.
F52882	F	-	29	No visible lesions.
	Group 3 - T-6054 (1.28 mg/kg)			
F52965	M	-	29	No visible lesions.
F52891	M	-	29	No visible lesions.
F52892	M	-	29	No visible lesions.
F52884	F	-	29	No visible lesions.
F52900	F	-	29	No visible lesions.
F52968	F	-	29	No visible lesions.

- Not applicable.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 4 - T-6054 (12.8 mg/kg)		
F52886	M	-	29	No visible lesions.
F52880	M	-	29	No visible lesions.
F52899	M	-	29	No visible lesions.
F52889	F	-	29	No visible lesions.
F52894	F	-	29	No visible lesions.
F52895	F	-	29	No visible lesions.
		Group 5 - T-6051 (10-cm x 10-cm Section)		
F52879	M	-	29	No visible lesions.
F52963	M	-	29	No visible lesions.
F52874	M	-	29	No visible lesions.
F52877	F	-	29	No visible lesions.
F52888	F	-	29	No visible lesions.
F52966	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
Group 1 (Control) - Sterile Water for Injection (0 mg/kg)					
Male	F52885	70.634	-	0.960	1.0
	F52873	75.513	16.417	0.866	1.6
	F52898	74.433	-	0.645	0.9
Female	F52967	62.517	-	0.777	2.1
	F52878	63.222	16.808	0.890	2.0
	F52883	75.418	-	0.618	2.5
Group 2 - T-6054 (0.128 mg/kg)					
Male	F52887	71.149	-	0.784	1.1
	F52893	64.537	13.509	0.836	0.6
	F52897	73.622	-	0.604	1.5
Female	F52901	71.908	14.610	0.640	1.7
	F52876	66.359	-	1.179	1.2
	F52882	77.959	-	1.337	1.6
Group 3 - T-6054 (1.28 mg/kg)					
Male	F52965	69.880	18.191	0.452	1.8
	F52891	65.695	-	0.525	1.1
	F52892	69.216	-	0.949	1.1
Female	F52884	65.907	-	0.590	1.2
	F52900	61.937	-	0.710	1.0
	F52968	67.347	12.513	0.866	1.3

- Not applicable.

Table 5 (Continued)
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g)			Bile Volume (mL)
		Liver	Kidneys	Dermal Application Site	
		Group 4 - T-6054 (12.8 mg/kg)			
Male	F52886	77.219	-	0.857	0.6
	F52880	59.985	12.254	0.634	0.8
	F52899	89.883	-	1.391	0.4
Female	F52889	69.288	-	0.953	0.5
	F52894	75.086	-	1.000	1.4
	F52895	59.174	15.603	0.635	0.9
		Group 5 - T-6051 (10-cm x 10-cm Section)			
Male	F52879	89.874	-	0.884	1.0
	F52963	71.814	-	1.535	0.6
	F52874	71.657	15.784	0.990	0.6
Female	F52877	71.284	-	1.154	0.6
	F52888	65.633	-	1.231	0.5
	F52966	68.719	15.855	0.898	1.5

- Not applicable.

APPENDIX A

Protocol Deviation
Protocol TP3016.AB
Protocol Amendment No. 1
Protocol Amendment No. 2

Protocol Deviation

Protocol	Actual Procedure
Page 7, 7. Experimental Design, C. Observation of Animals, (2) Reading of Dermal Irritation, Second Sentence. Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8.	The Day 8 erythema score was inadvertently not recorded for one Group 4 female (No. F52889).

This deviation is not considered to have had an adverse effect on the outcome of the study.

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Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

Date:

December 13, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-133

Phone 608 241 4471
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704
FAX 608 241 7227

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

HWI No.	6329-133
Test Materials	1. T-6054 2. T-6051
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	December 28, 1994
Experimental Termination Date	January 25, 1995
Draft Report Date	March 8, 1995

1. Study
Single-Dose Dermal Absorption/Toxicity Study in Rabbits

2. Purpose
To assess the systemic absorption and toxicity and relative skin irritancy of test materials when applied to the skin of rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Materials

A. Identification
1. T-6054
2. T-6051

B. Physical Description
1. (To be documented in the raw data)
2. (To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test and control materials will be taken for this study.

The test and control material reserve samples will be stored at HWI in a freezer set to maintain a temperature of -20°C ±10°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

F. Retention
Any unused test materials will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Distilled water

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this manufactured material is considered to be adequate for the purposes of this study.

D. Storage Conditions
Room temperature

E. Reserve Samples
See Section 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
15 males and 15 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0*	3	3
2	T-6054	0.128	3	3
3	T-6054	1.28	3	3
4	T-6054	12.8	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)

(2) Preparation of Exposure Area
On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair with an electric clipper. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration
All animals will receive a single administration of the respective test or control material. The day of treatment will be designated as Day 1. The respective doses for the animals in Groups 1, 2, 3, and 4 will be based on the animal's body weight just before administration and spread onto the area of exposure in a thin and uniform layer. The Group 1, 2, 3, and 4 materials will be applied undiluted. The Group 5 material will be applied as a 10.0-cm x 10.0-cm piece of the test material moistened with distilled water. The area of application (Groups 1-5) will be covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during the 24-hour application period.

(4) Reason for Route of Administration
The dermal route is a potential route of exposure in humans.

(5) Removal of Test Material
Approximately 24 hours after test or control material application the bandages and collars will be removed and the residual test material will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Clinical Observations
For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days. Observations may be extended when directed by the study director.

(2) Reading of Dermal Irritation
Before test or control material administration the initial dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8. Individual dermal irritation records will be maintained for each animal.

(3) Body Weights
For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day)

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 8, 15, and 22. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or

sacrificed at the time of necropsy (Day 29). The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

D. Pathology

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section D. Pathology, (3) Sample Collection, will be collected. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice
At termination of the experimental phase (Day 29), surviving animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded.

(3) Sample Collection
The sites of test and control material application will be washed with lukewarm tap water prior to the necropsy procedure. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necopsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

The tissues (liver, bile, dermal application site, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.C.(4).(c). The Sponsor is responsible for the retention and disposition of the samples.

E. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings/gross pathology report

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Anatomical pathology records
Sample collection records
Shipping records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

12-15-94
Date

Steven M. Glaza

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

12-13-94
Date

Stacy Thiede

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12-13-94
Date

(6329-133.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema

0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia

0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation

0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring

0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

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Single-Dose Dermal Absorption/Toxicity Study
of T-6054 and T-6051 in Rabbits

HWI 6329-133

Sponsor

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Medical Department
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P.O. Box 33220
St. Paul, MN 55133-3220

Contractor

Hazleton Wisconsin, Inc
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative

John L. Butenhoff, PhD

Study Director

Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective December 23, 1994

In order to obtain a measurable amount of test material (T-6054) for application in Groups 2, 3, and 4, the test material will be diluted with sterile water for injection and applied at a common dose volume of .01 mL/kg. Modify the following two sections of the protocol (protocol amendment items #1 and #2) to indicate these changes.

1. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. Add the following shaded additions to this section:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0*	3	3
2	T-6054	0.128***	3	3
3	T-6054	1.28***	3	3
4	T-6054	12.8***	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)
*** To be administered at a dose volume of .01 mL/kg

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Amendment No. 1

2. Page 6, 7. Experimental Design; B. Dose Administration; (3) Dose Administration. Delete the fourth sentence in this section and then add the following as the third and fourth sentences to this section.

> The control material (Group 1) will be applied undiluted. The dose for each animal in Groups 2, 3, and 4 will be diluted with sterile water for injection and applied at a dose volume of .01 mL/kg.

Effective December 28, 1994

Sterile water for injection will replace distilled water as the control material based on the fact that sterile water for injection will also be the vehicle in the test mixtures for Groups 2, 3, and 4 (see protocol amendment items #1 and #2). Modify the following three sections of the protocol to indicate this change.

3. Page 4, 6. Control Material; A. Identification. Replace *distilled water* with the following:

> Sterile Water for Injection

4. Page 4, 6. Control Material; D. Storage Conditions. Replace *Room temperature* with the following:

> Refrigerated

5. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. Modify the table in this section with the following shaded change:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Sterile water	0*	3	3
2	T-6054	0.128***	3	3
3	T-6054	1.28***	3	3
4	T-6054	12.8***	3	3
5	T-6051	**	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered as a 10.0-cm x 10.0-cm piece of test material (fabric)
*** To be administered at a dose volume of .01 mL/kg

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

1-19-95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

1-12-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

1.12.95
Date

(6329-133.Aml.dsk2)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study of
T-6054 and T-6051 in Rabbits

HWI 6329-133

Sponsor	Contractor
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 2

This amendment modifies the following portions of the protocol:

Effective January 24, 1995

At the request of the Sponsor, the weights of tissues collected and the volume of bile collected will be documented in the raw data. These weights and volumes will be included with the sample shipment. Modify the following sections of the protocol to include these additions.

1. Page 8, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first paragraph of this section with the following underlined addition:

   The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.

2. Page 9, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the second paragraph of this section with the following underlined addition:

   The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

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Fax 608 241 7227

Amendment No. 2

3. Page 9, 8. Report. Add the following to this section:

Individual animal tissue weights and bile volumes

PROTOCOL AMENDMENT APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

2/15/95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

2-6-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

2-7-95
Date

(6329-133.Am2.dsk2)

9.1.2 Analytical protocol AMDT-013195.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6051 and T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-133

Study Number: AMDT-013195.1
Test Substance: FC-129 (T-6051 and T-6054)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson — Date
Study Director

John L. Butenhoff 10/30/95
John Butenhoff, PhD — Date
Sponsor Representative

1.0 PURPOSE

This study is designed to provide information as to whether FC-129 (T-6051 and T-6054) is dermally absorbed. The analytical aspect of this study is to determine fluorine-containing compounds (biotransformation products) in the tissue and serum of rabbits at various times post dose dermal application of FC-129.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4-liver), Sigma Chemical Company (2.1.2, 2.1.4-serum)

2.3 Number of Test and Control Samples: Tissues and fluid from 24 test animals and 6 control animals. Tissues and fluids include liver, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), $-20 \pm 10°C$ (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-133), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (see Methods--next section) is the appropriate analysis to present definitive data for fluorine in the liver. To confirm the identity of fluorine-containing compounds present in liver (if any at 28 days) and serum at various intervals, electrospray mass spectrometry may be selected as one of the analytical techniques employed. Not all of the tissues and fluid samples will be analyzed. When sufficient data has been collected to meet the objectives of the study in the opinion of the Study Director, analysis will cease.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include regression analysis of serum concentrations with time and averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D Johnson 10/3/95
Group Leader Date

Gale Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 10-31-95
Date

Quality Assurance 10-31-95
Date

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.
1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.
1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 Reagents

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step 9.12 of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{171 \text{ mg liver}^{*} / 1 \text{ ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 **Calibration**

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 **Storage Conditions for Standards**

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 **Storage Conditions for Standards**

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in 8.3.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W______	FC-95 approx 1 ppm actual ppm #W______	FC-95 approx. 5 ppm actual ppm #W______	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
1		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:		Date & Initials
Pipet 1 mL of Liver Solution		
Pipet 100 uL of 12 ppm Internal Standard	Std. #	
Vortex 15 sec		
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer		
Pipet 5 mL of Ethyl Acetate		
Vortex 20-30 sec		
Shake 20 min		
Centrifuge 20-25 min		
Remove a 4 mL aliquot of organic layer		
Blow down to near dryness (<0.25 mL) with N_2		
Add 1 m: of 1:1 Acetonitrile/H_2O	TN#	
Vortex 15 sec		
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial		

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 6/6/95
Date

Quality Assurance 6/6/95
Date

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 Scope: This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES^-.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES^-, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 **Using the Autosampler**

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

9.3.3.1 Push the sample button

9.3.3.2 Set sample loop size = 100 uL

9.3.3.3 Set inject/sample = 2

9.3.3.4 Set Cycle time = 0

9.3.3.5 Name the file: Livers

9.3.3.6 Identify the tray used

9.3.3.7 Add the samples to Queue by pressing "Enter"

9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 **Precision and Accuracy**

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 **Other Validation Parameters**

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

Revision Number: 0 **Revision Date:** None

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Von Burkett 9-27-95
Quality Assurance Date

Software: IBM MS Word, 6.0
Affected Documents: **AMDT-EP-26,** Operation and Maintenance of the Skalar Segmented Flow Analyzer

1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps

4.1.2 Autopipets, oxford or equivalent with plastic tips

4.1.3 Polypropylene volumetric flasks, 100 mL

4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.

4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent

4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.

4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.

4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.

4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.

4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 **Preparation of Calibration Standards**

8.1.1 Prepare calibration standards as in section 4.3.

8.2 **Calibration**

8.2.1 The standards are analyzed at the beginning of the run.

8.3 **Storage Conditions for Standards**

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 **Start Up Procedure**

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.

9.1.2 Put the fluoride electrodes in the electrode chamber.

9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.

9.1.4 Put the reagent-lines in the appropriate bottles.

9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**

9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 **Starting a Run**

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.

9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.

9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.

9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.

9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under **9.2.1** (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-M-1, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor | Original to: Study Director | Copies to: QAU Files

Study Title: **Single-dose Dermal Absorption/Toxicity Study of T-6051 and T-6054 in Rabbits**

Study Number: AMDT-013195.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/12/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95
QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-013195.1, HWI 6329-133
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group*

	µg		Std. Dev.
Control Group	**16.2**	**±**	**5.5**
0.128 mg/kg dose (T6054)	**11.1**	**±**	**2.0**
1.28 mg/kg dose (T6054)	**17.1**	**±**	**2.6**
12.8 mg/kg dose (T6054)	**45.2**	**±**	**20.5**
Fabric Exposure (T6051)	**14.7**	**±**	**2.7**

***Calculated as the mean of triplicate samples from each of three male and three female rabbits.**

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
liver blank-3		0.230		0.140			
liver spike-4	83%	0.950		0.132			
liver spike-5	83%	1.06		0.118			
liver spike-6	102%	1.28		0.121			
liver spike-7	94%	1.00		0.142			
liver spike-8	84%	2.34		0.108			
liver spike-9	83%	2.49		0.101			
liver blank-4		0.328		0.116			
F52873-1		0.245		0.121	74.2		
F52873-2		0.334	0.303	0.107	74.2	22.4	0.0
F52873-3		0.329		0.104	74.2		
F52885-1		0.449		0.150	69.2		
F52885-2		0.222	0.326	0.114	69.2	22.5	0.0
F52885-3		0.306		0.108	69.2		
F52898-1		0.139		0.150	73.3		
F52898-2		0.131	0.124	0.148	73.3	9.11	0.0
F52898-3		0.103		0.146	73.3		
liver blank-1		0.228		0.152			
liver blank-2		0.298		0.134			
liver spike-1	91%	1.36		0.101			
liver spike-2	95%	0.995		0.145			
F52878-1		0.286		0.105	70.2		
F52878-2		0.230	0.231	0.150	70.2	16.2	0.0
F52878-3		0.176		0.151	70.2		
F52883-1		0.211		0.115	73.8		
F52883-2		0.242	0.211	0.107	73.8	15.6	0.0
F52883-3		0.179		0.135	73.8		
F52967-1		0.158		0.144	61.5		
F52967-2		0.194	0.184	0.116	61.5	11.3	0.0
F52967-3		0.200		0.108	61.5		
F52887-1		0.252		0.108	70.6		
F52887-2		0.189	0.205	0.128	70.6	14.5	0.128
liver blank-1		0.281		0.129			
liver spike-1	69%	0.968		0.108			
liver spike-2	85%	0.973		0.132			
liver spike-3	83%	0.875		0.143			
liver spike-4	109%	1.29		0.128			
liver spike-5	101%	1.11		0.138			
liver spike-6	101%	1.26		0.121			
F52887-3		0.173		0.148	70.6		
F52893-1		0.168		0.133	63.5		
F52893-2		0.147	0.163	0.135	63.5	10.4	0.128
F52893-3		0.175		0.135	63.5		

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
F52897-1		0.156		0.134	72.4		
F52897-2		0.174	0.169	0.113	72.4	12.3	0.128
F52897-3		**0.179**		0.123	72.4		
F52876-1		**0.117**		0.149	65.2		
F52876-2		**0.155**	0.139	0.119	65.2	9.09	0.128
F52876-3		**0.146**		0.138	65.2		
F52882-1		0.112		0.146	76.1		
F52882-2		0.154	0.145	0.130	76.1	11.0	0.128
F52882-3		0.169		0.112	76.1		
F52901-1		0.126		0.140	70.5		
F52901-2		0.127	0.132	0.143	70.5	9.29	0.128
F52901-3		0.142		0.112	70.5		
Liver blk 1		0.331		0.112			
Liver blk 2		0.218		0.137			
Liver blk 1		1.22		0.146			
Liver blk 2		0.322		0.159			
Liver blk 3		0.280		0.139			
Liver spk-1	92%	1.10		0.127			
Liver spk-2	89%	0.869		0.156			
Liver spk-3	83%	0.864		0.145			
F52965-1		0.235		0.125	68.7		
F52965-2		0.205	0.219	0.163	68.7	15.0	1.28
F52965-3		0.216		0.151	68.7		
F52891-1		0.239		0.114	64.5		
F52891-2		0.197	0.225	0.144	64.5	14.5	1.28
F52891-3		0.239		0.128	64.5		
F52892-1		0.333		0.132	68.7		
F52892-2		0.270	0.292	0.134	68.7	20.1	1.28
F52892-3		0.273		0.102	68.7		
F52884-1		0.206		0.146	65.0		
F52884-2		0.255	0.231	0.101	65.0	15.0	1.28
F52884-3		0.230		0.135	65.0		
F52968-1		0.299		0.145	66.6		
F52968-2		0.261	0.267	0.116	66.6	17.8	1.28
F52968-3		0.242		0.121	66.6		
F52900-1		0.335		0.147	61.4		
F52900-2		0.390	0.328	0.115	61.4	20.1	1.28
F52900-3		0.259		0.115	61.4		
F52880-1		0.487		0.117	59.6		
F52880-2		0.548	0.538	0.142	59.6	32.1	12.8
F52880-3		0.579		0.138	59.6		
F52886-1		0.611		0.137	75.3		
F52886-2		0.728	0.610	0.114	75.3	46.0	12.8
F52886-3		0.490		0.113	75.3		
F52899-1		0.428		0.103	89.1		
F52899-2		0.366	0.378	0.115	89.1	33.7	12.8
F52899-3		0.340		0.126	89.1		

FC129 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
liver blank 1		0.265		0.125			
liver blank 2		0.327		0.111			
liver blank 3		0.220		0.123			
liver spike-1	87%	0.921		0.143			
liver spike-2	0.7786	**1.05**		0.112			
liver spike-3	0.7852	**0.823**		0.144			
liver spike-4	**0.9656**	**1.06**		0.138			
liver spike-5	**0.8486**	**0.859**		0.149			
liver spike-6	82%	0.978		0.127			
F52889-1		0.812		0.114	68.7		
F52889-2		0.896	1.10	0.113	68.7	75.4	12.8
F52889-3		1.59		0.141	68.7		
F52894-1		0.835		0.127	74.0		
F52894-2		0.794	0.849	0.119	74.0	62.8	12.8
F52894-3		0.917		0.118	74.0		
F52895-1		0.307		0.151	58.1		
F52895-2		0.523	0.368	0.109	58.1	21.4	12.8
F52895-3		0.276		0.154	58.1		
F52874-1		0.331		0.144	69.7		
F52874-2		0.249	0.251	0.122	69.7	17.5	Fabric
F52874-3		0.173		0.145	69.7		
F52879-1		0.168		0.134	89.1		
F52879-2		0.194	0.182	0.105	89.1	16.3	Fabric
F52879-3		0.185		0.147	89.1		
F52963-1		0.216		0.130	70.2		
F52963-2		0.175	0.182	0.123	70.2	12.8	Fabric
F52963-3		0.154		0.135	70.2		
F52877-1		0.222		0.119	69.9		
F52877-2		0.190	0.191	0.118	69.9	13.3	Fabric
F52877-3		0.160		0.141	69.9		
F52888-1		0.158		0.144	64.2		
F52888-2		0.211	0.170	0.104	64.2	10.9	Fabric
F52888-3		0.142		0.139	64.2		
F52966-1		0.321		0.123	67.6		
F52966-2		0.277	0.255	0.131	67.6	17.3	Fabric
F52966-3		0.167		0.132	67.6		
Liver spike-7	60%	0.881		0.102			
Liver spike-8	71%	1.05		0.103			
Liver spike-9	75%	0.775		0.146			
Liver spike-10	98%	0.990		0.150			
Liver spike-11	103%	1.15		0.136			
Liver spike-12	101%	1.16		0.133			
Liver spike-13	78%	2.16		0.110			
Liver spike-14	107%	2.46		0.131			
Liver spike-15	102%	2.39		0.130			

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

A-1
Contains pages
A-1 through A-
10-31-95
D. Christenson

Study: Single Dose Dermal Absorption
Protocol Number: TP3016.AB
Test Material: T-6054 & T-6051 in Rabbits (FC-129)
Matrix: Liver
R Squared Value: 0.9889
Response Factor Amount: 1.15E-05
Analyst: DLC
Date: 4/3/95
Method: AMDT-M-4
Instrument: Fisons VG 2000 Electrospray MS
LABBASE FILE 040395D

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g *	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: Sterile Water 2.0 mL/kg	F52883	N/D			N/D		N/D
	F52898	N/D			N/D		N/D
	F52885	N/D			N/D		N/D
	F52967	N/D			N/D		N/D
	F52873	N/D			N/D		N/D
	F52878	N/D			N/D		N/D
Group 2: 0.128 mg /kg	F52893	N/D			N/D		N/D
	F52887	N/D			N/D		N/D
	F52901	N/D			N/D		N/D
	F52876	N/D			N/D		N/D
Group 3: 1.28 mg/kg	F52892	11413	1.0139	1	0.1033	68.707	0.007
	F52891	14922	1.0615	1	0.1290	64.487	0.008
	F52965	8191	1.1217	1	0.0670	68.667	0.005
	F52884	12572	1.0881	1	0.1060	64.989	0.007
Group 4: 12.8 mg/kg	F52895	51584	1.117	1	0.4237	58.094	0.025
	F52899	58481	1.1894	1	0.4511	89.078	0.040
	F52894	127876	1.0236	1	1.1462	73.983	0.085
	F52880	58961	1.0931	1	0.4949	59.624	0.030
	F52886	84958	1.0915	1	0.7142	75.341	0.054
	F52889	93984	1.0615	1	0.8124	68.670	0.056
Group 5: Fabric	F52879	16734	1.2566	1	0.1222	89.125	0.011
	F52877	26249	1.0106	1	0.2383	69.913	0.017
	F52874	N/D			N/D		N/D
	F52963	9735	1.0772	1	0.0829	70.161	0.006
	F52888	N/D			N/D		N/D

* **The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.**

HWI # 6329-133

Study:	Single Dose Dermal Absorption
Protocol Number:	TP3016.AB
Test Material:	T-6054 & T-6051 in Rabbits (FC-129)
Matrix:	Liver
R Squared Value:	0.9946
Response Factor Amount:	9.10E-06
Analyst:	DLC
Date:	4/4/95
Method:	AMDT-M-4
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE FILE	040495B

Group Dose	Sample #	Ion Count Area	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg
Group 2: **0.128 mg /kg**	F52897	18736	1.1033	1	0.1236	72.433	0.009
	F52882	N/D	1.1025	1	N/D	76.060	N/D
Group 3: **1.28 mg/kg**	F52900	16432	1.3161	1	0.0909	61.356	0.006
	F52968	27181	1.2011	1	0.1648	66.642	0.011
Group 5: **Fabric ***	F52966	8521	1.1457	1	0.0542	67.632	0.004

*** Administered as a 10.0 cm x 10.0 cm piece of test fabric**

**** The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.**

Replot

A-3

Method DLCLIV
Sample DLCLIV
Operator DLC

FILE: 040495B

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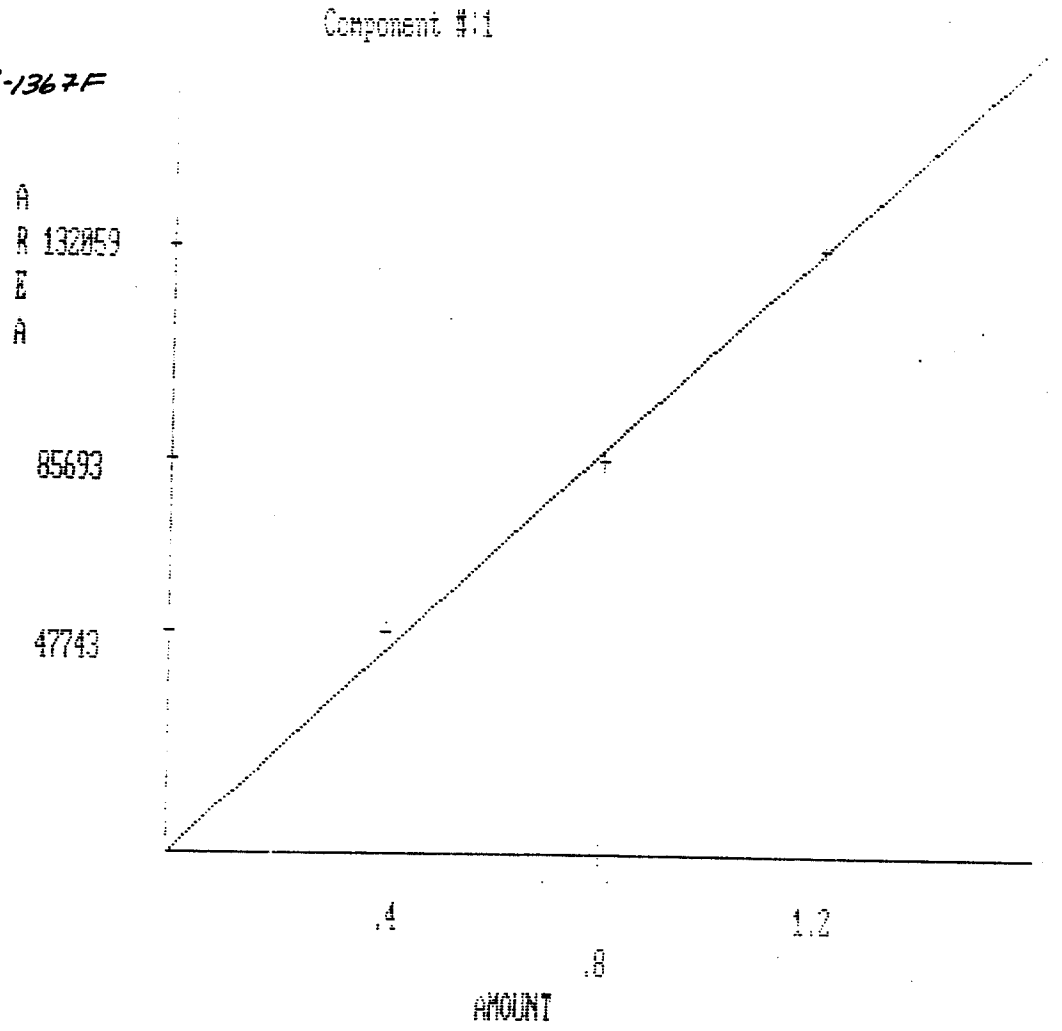
6329-143 FC-1367F
6329-133



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2	0.8000	85693
3	1.2000	132059

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Amount = 9.1012E-06 * Area + 0.0000E+00
R squared = 0.9946

FILE: 040395D

Keplot

A-4

HWL 6329-133

FC-129

Method DLCLIV
Sample DLCLIV
Operator DLC

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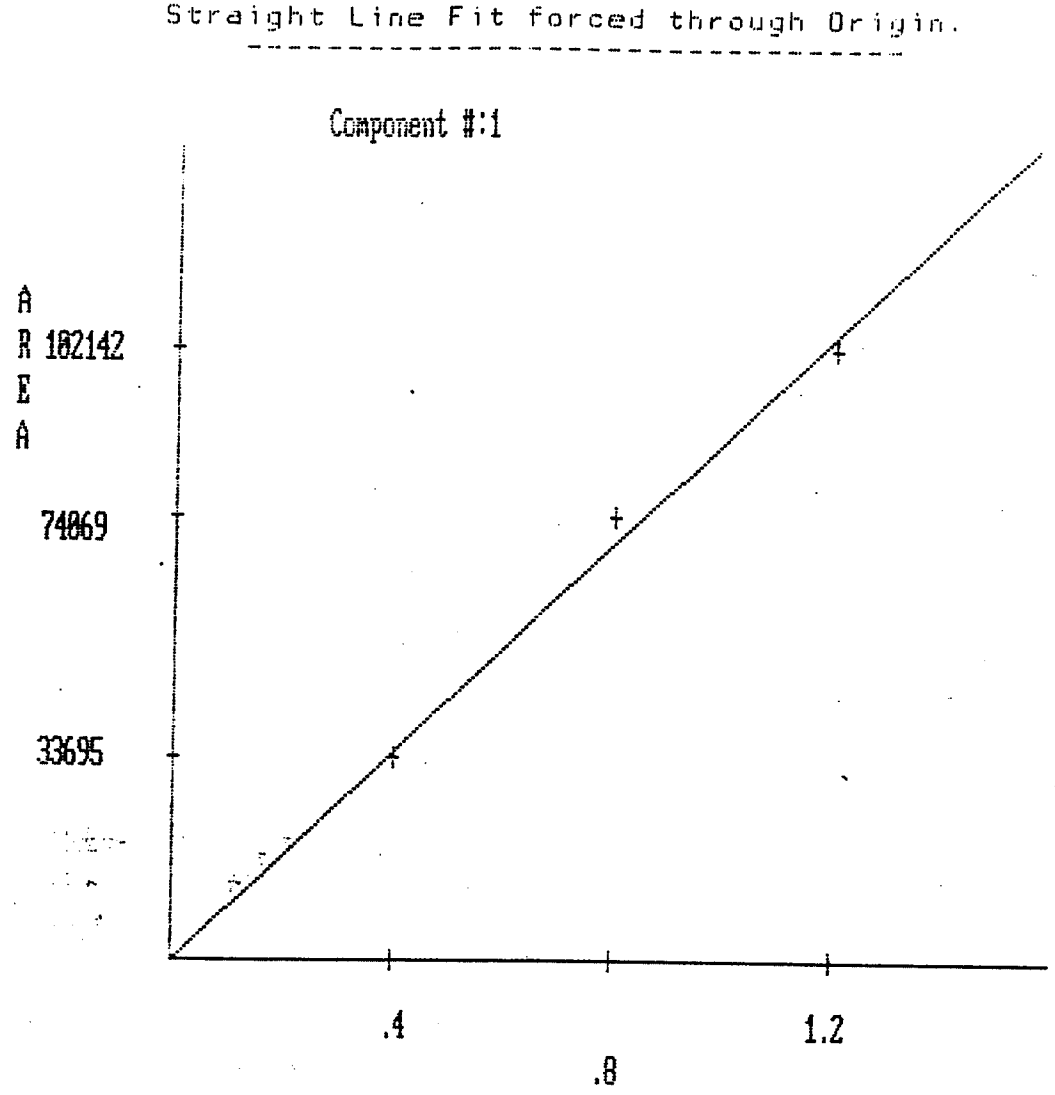
DLC Initial

6/22/95 Date

Exact Copy of Original



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R squared = 0.9889

HwI 6329-133

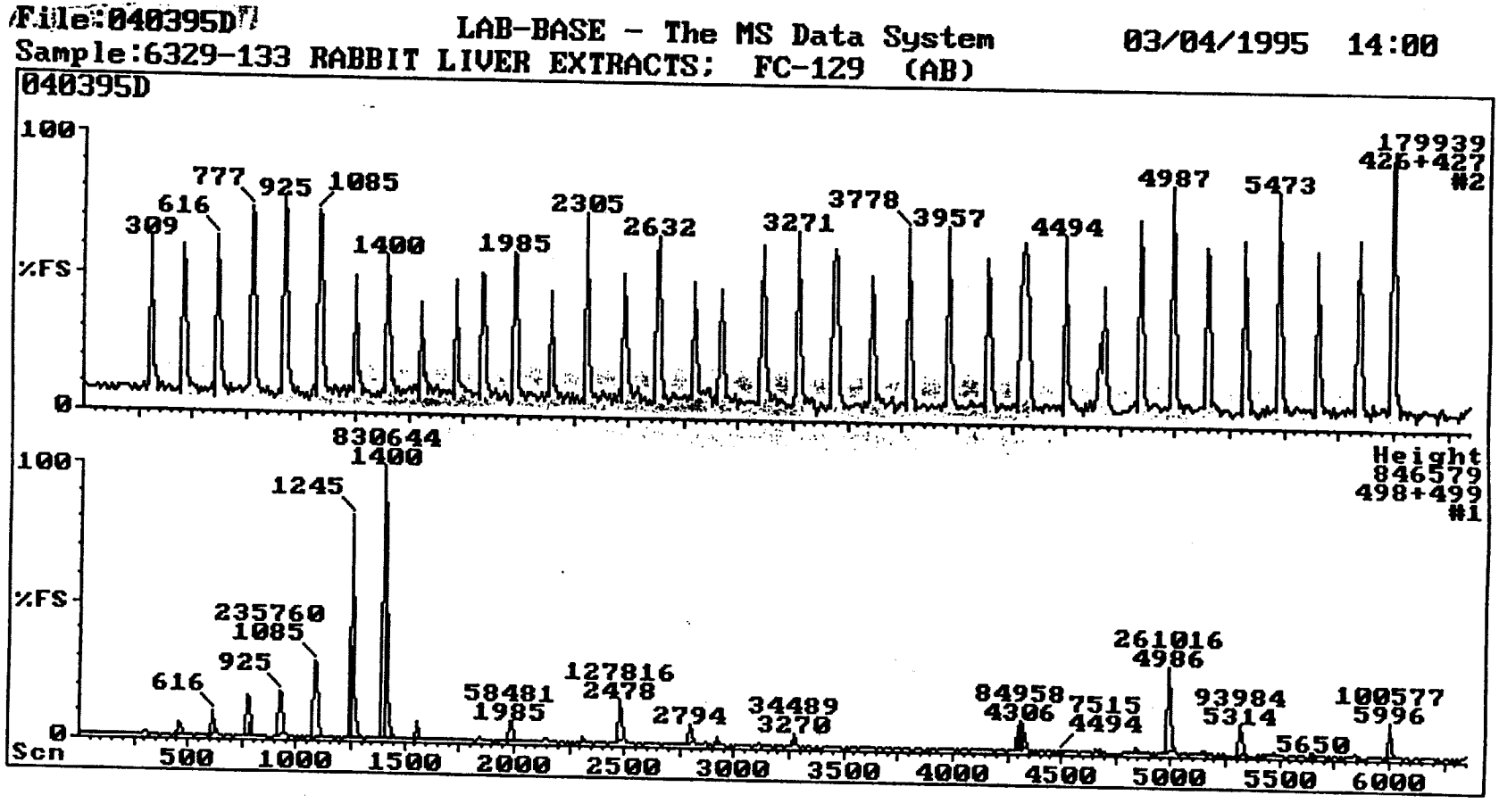
FC-129

A-6

Exact Copy of Original

DLC
Initial

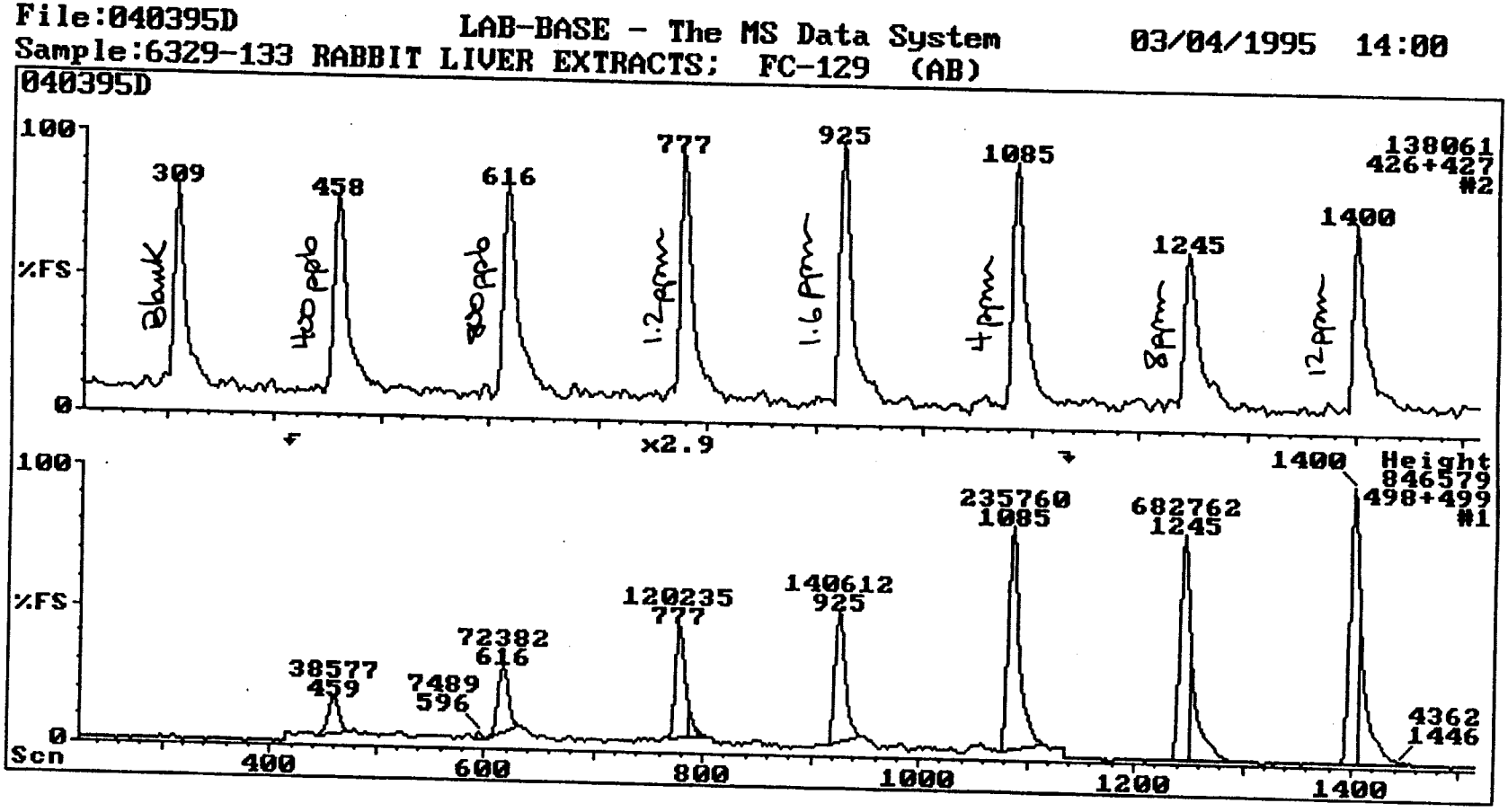
6/22/95
Date



000369

A-10 10-1 (82)
A-7

Beef liver extracts for std. curve



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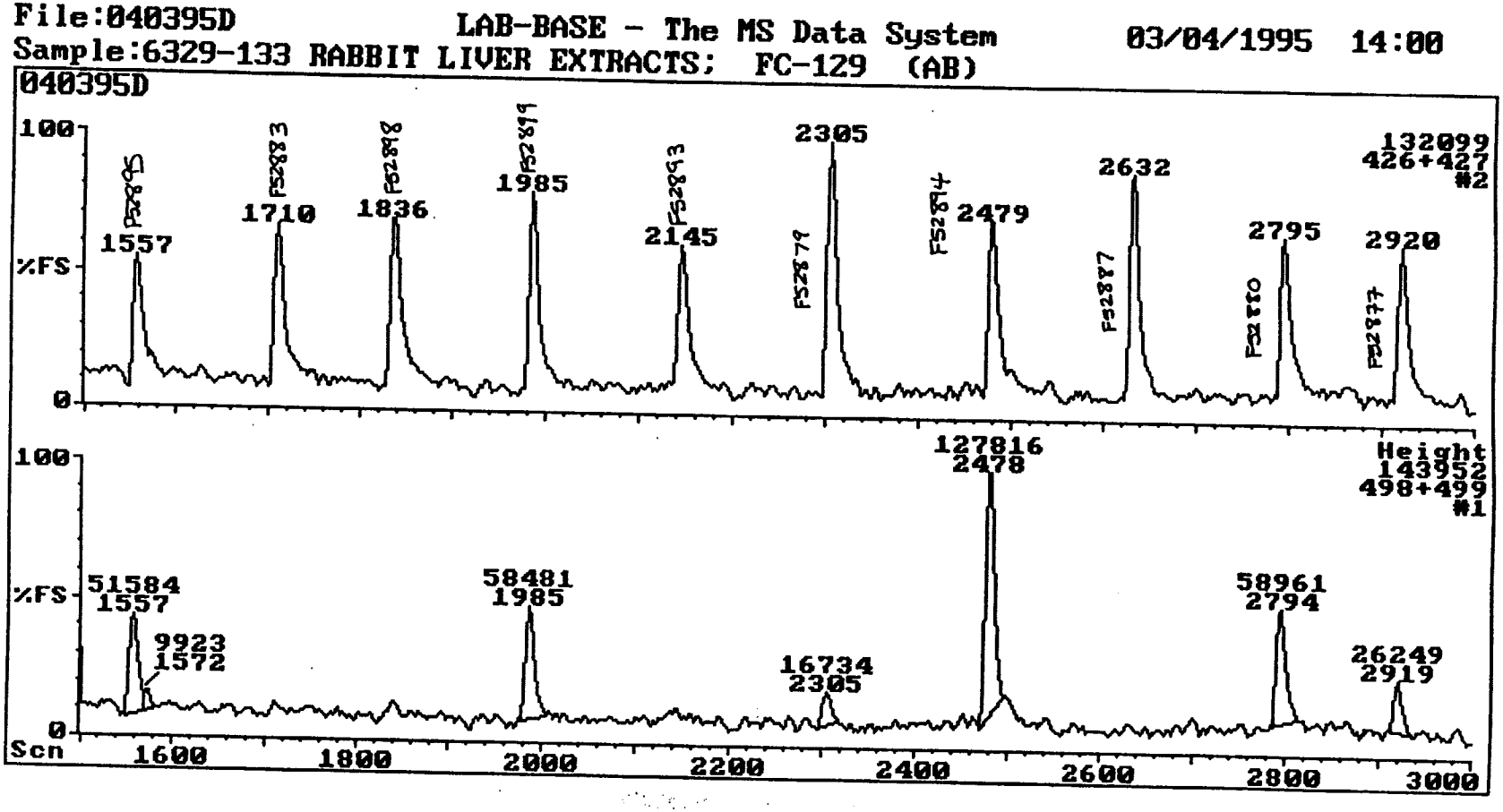
A-8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2145
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2479
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2795
2920
132099
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1985
16734
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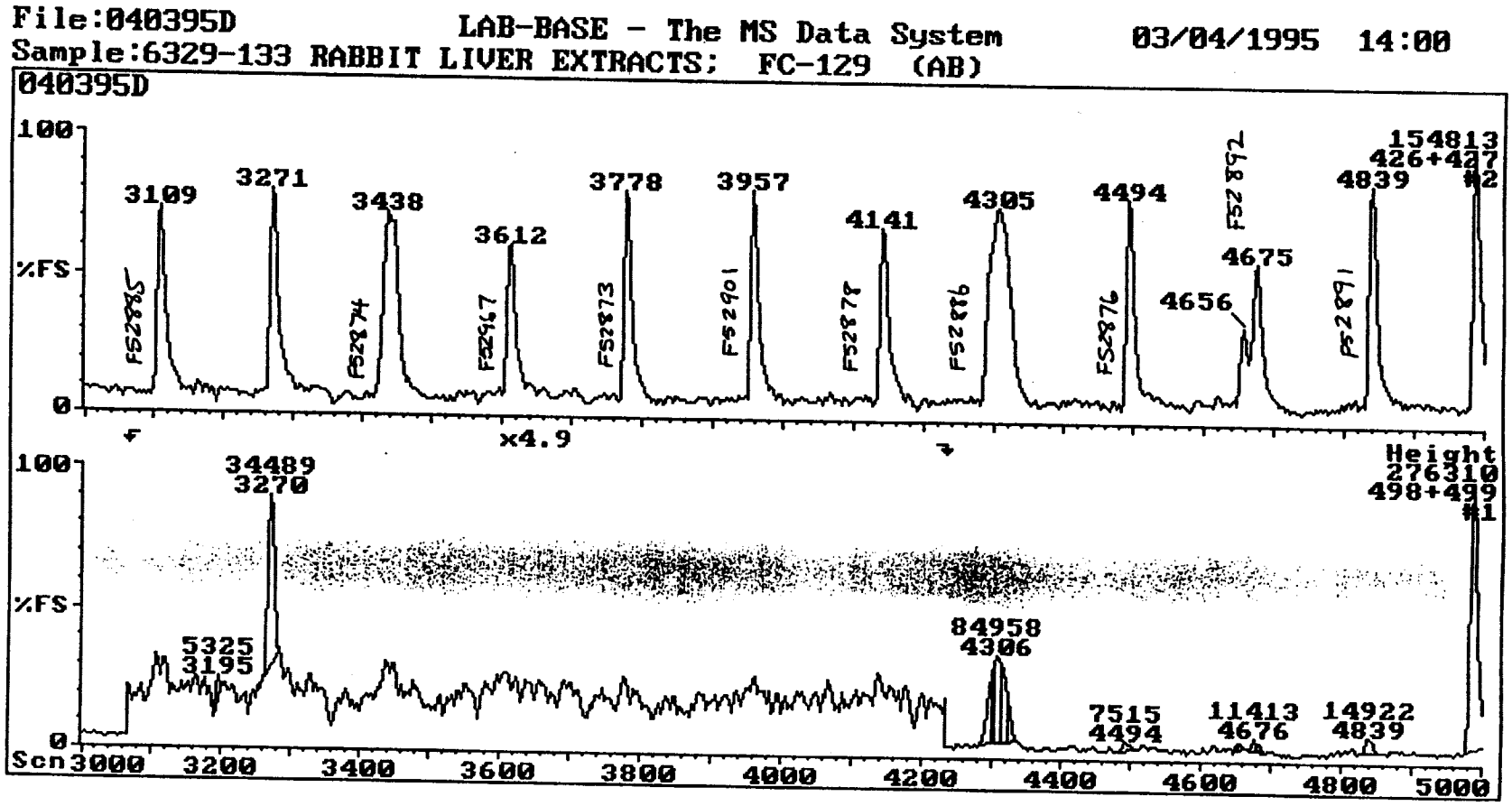
A-9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FS2891
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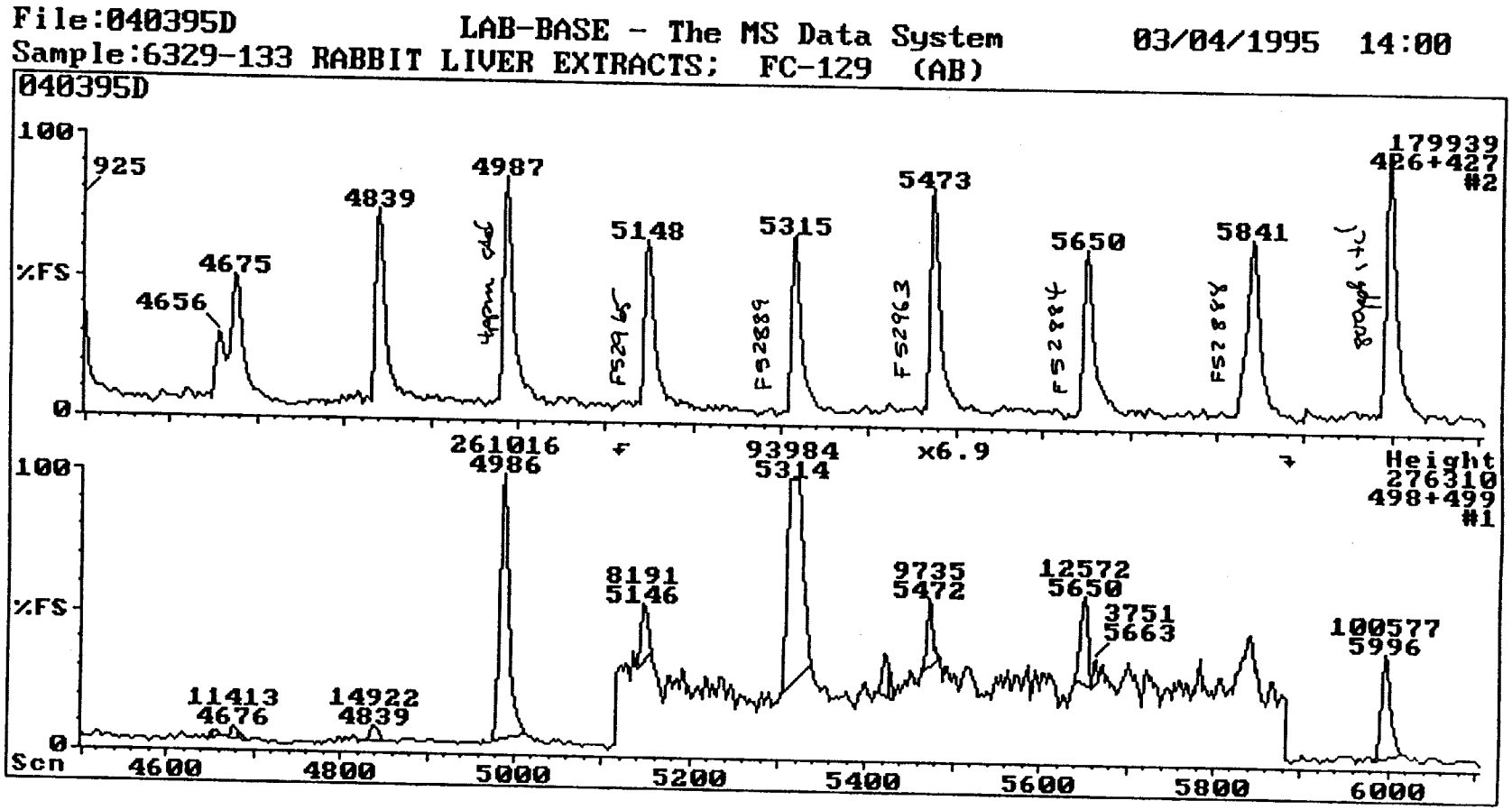
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5650
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426+427
#2
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93984
5314
x6.9
Height
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498+499
#1
8191
5146
9735
5472
12572
5650
3751
5663
100577
5996
11413
4676
14922
4839
Scn
4600
4800
5000
5200
5400
5600
5800
6000

A-11

DLC

HWI 6329-133 & 6329-143

Copy of Original

DLC — Initial

6/2295 — Date

6329-133 Animals Analyzed

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F52900
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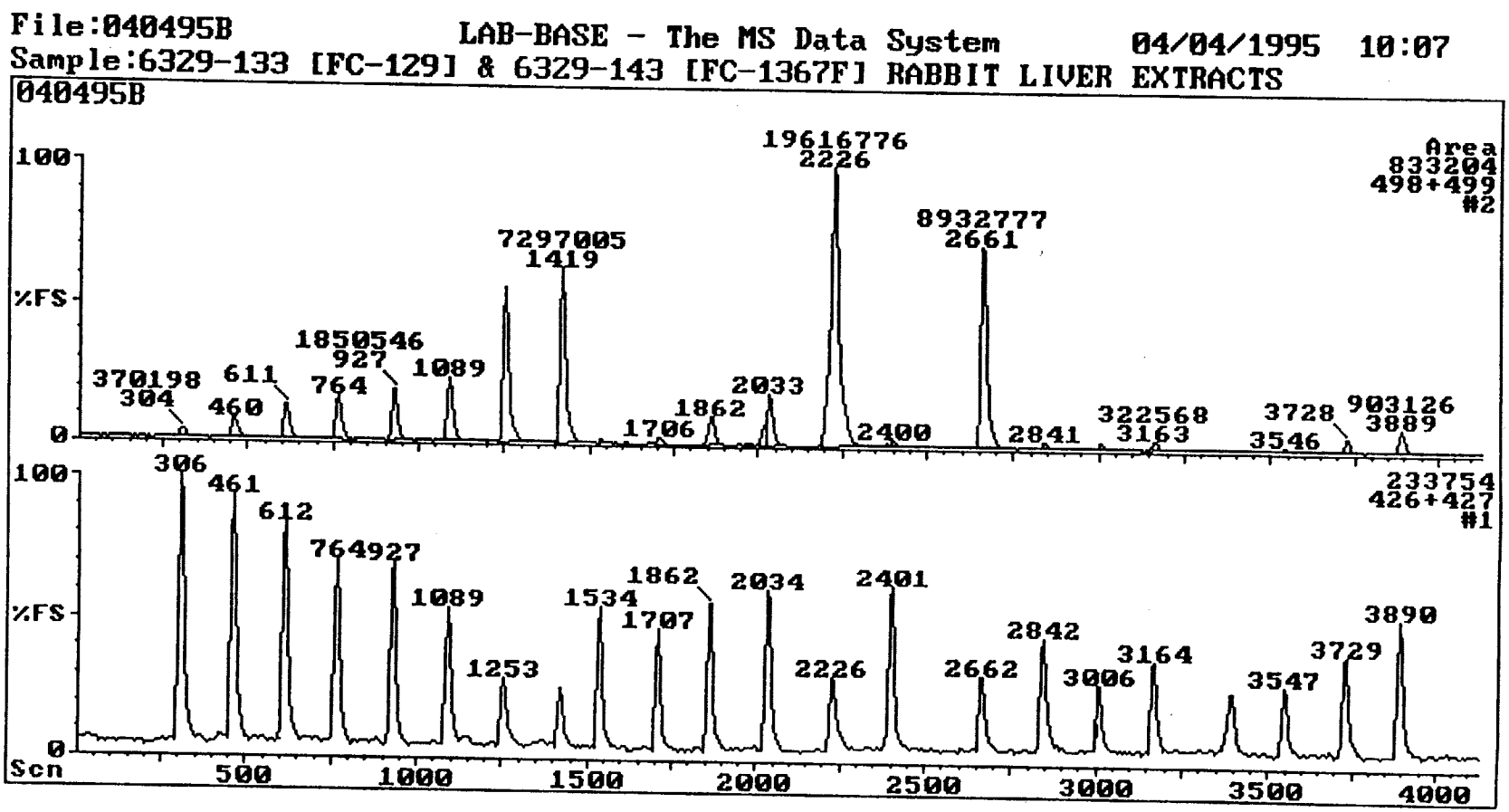
F52882
F52966



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A-45
A-12
10-18-95
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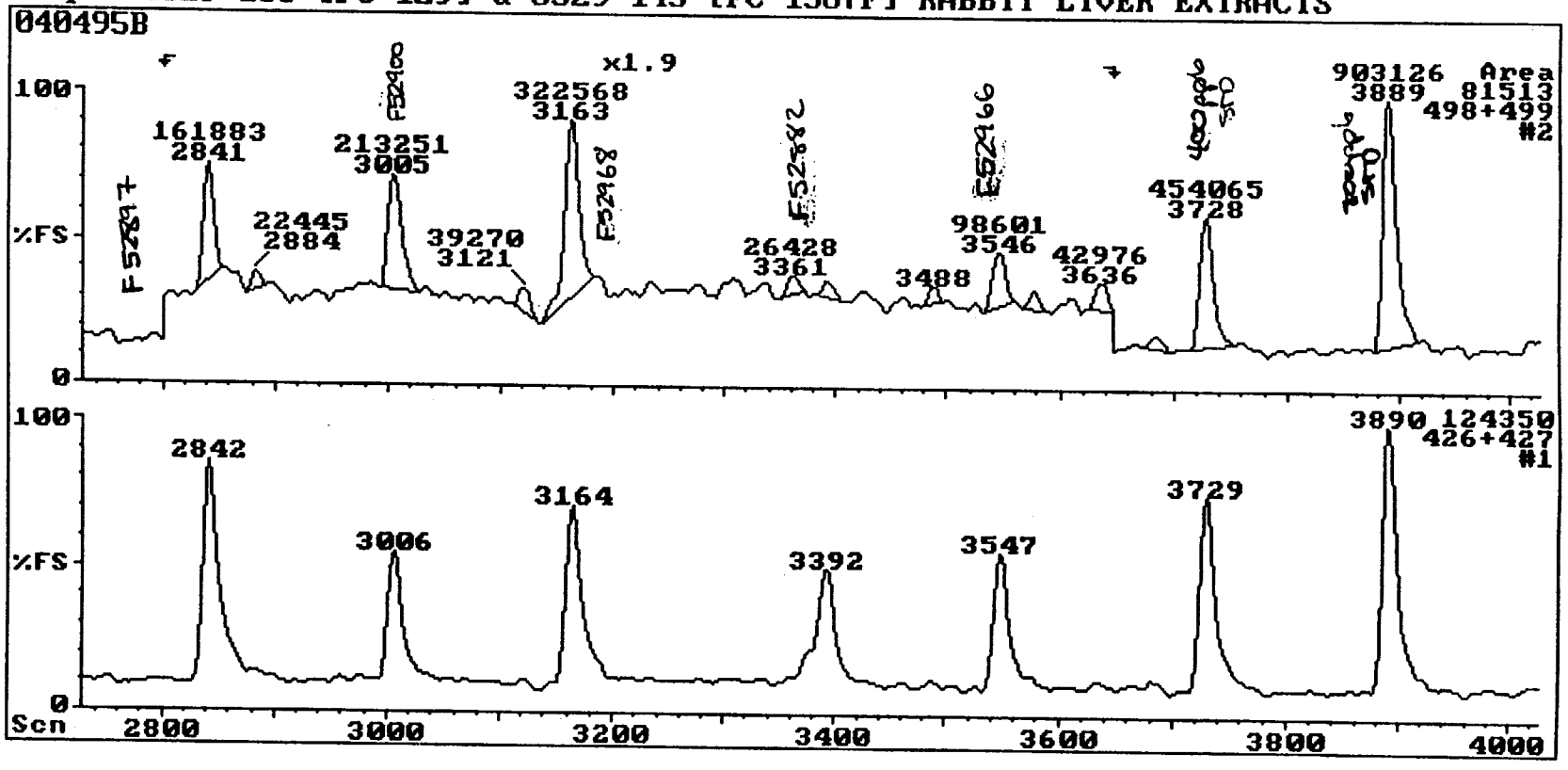

000375

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in section 6.0 and therefore is not discussed in detail.

DDW 7/18/95
Skalar Data

RE: 6329-133 LIVER SAMPLES
AMDT 13195.1
Date of Analysis: 3/28, 3/29 and 3/30/95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using between 0.1 and 0.2 grams of the liver. The gas is collected in 1.0 mL of 1:1 TISAB/Milli-Q water then an additional 2 mL of 1:1 TISAB/Milli-Q is added to allow for sufficient volume for Skalar analysis. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Deborah D Wright

SUMMARY of 6329-133
LIVER SAMPLES
AMDT 013195.1

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	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue (ug)

** This is only a partial study. All samples were not saved from the Dohrmann analysis. DDW

	F52884-1	ND	3.0	0.1463	ND		64.9889	ND	
	F52884-2	ND	3.0	0.1011	ND	ND	64.9889	ND	ND
	F52884-3	ND	3.0	0.1345	ND		64.9889	ND	
	F52891-1	ND	3.0	0.1140	ND		64.4869	ND	
	F52891-2	ND	3.0	0.1443	ND	ND	64.4869	ND	ND
	F52891-3	ND	3.0	0.1284	ND		64.4869	ND	
GROUP 3	F52892-1	0.02	3.0	0.1322	0.43	0.20	68.7067	29	15
Dose Level : 1.28 mg/kg	F52892-2	ND	3.0	0.1342	ND		68.7067	ND	
	F52900-1	ND	3.0	0.1474	ND		61.3555	ND	
	F52900-2	ND	3.0	0.1153	ND	ND	61.3555	ND	ND
	F52900-3	ND	3.0	0.1150	ND		61.3555	ND	
	F52965-1	0.02	3.0	0.1246	0.40	0.36	68.6665	28	25
	F52965-3	0.02	3.0	0.1510	0.31		68.6665	21	
	F52968-1	ND	3.0	0.1448	ND		66.6415	ND	
	F52968-2	ND	3.0	0.1159	ND	ND	66.6415	ND	ND
	F52968-3	ND	3.0	0.1211	ND		66.6415	ND	

Stolar Oct

SUMMARY of 6329-133
LIVER SAMPLES
AMDT 013195.1

	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F-	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue
	F52889-1	0.04	3.0	0.1139	1.17		68.6699	80	
	F52889-2	0.05	3.0	0.1125	1.34	1.56	68.6699	92	107
	F52889-3	0.10	3.0	0.1410	2.18		68.6699	149	
	F52894-1	0.05	3.0	0.1272	1.20		73.9834	89	
GROUP 4	F52894-2	0.06	3.0	0.1183	1.43	1.27	73.9834	105	94
Dose Level : 12.8 mg/kg	F52894-3	0.05	3.0	0.1185	1.17		73.9834	87	
	F52895-1	0.03	3.0	0.1513	0.62		58.0944	36	
	F52895-2	0.03	3.0	0.1091	0.87	0.65	58.0944	51	38
	F52895-3	0.02	3.0	0.1537	0.44		58.0944	26	
	F52874-1	0.02	3.0	0.1438	0.46		69.6893	32	
	F52874-2	0.02	3.0	0.1221	0.50	0.44	69.6893	35	31
	F52874-3	0.02	3.0	0.1454	0.35		69.6893	25	
GROUP 5	F52879-1	0.02	3.0	0.1338	0.34	0.17	89.1246	30	16
Dose Level : 10.0 cm x 10.0 cm fabric	F52879-2	ND	3.0	0.1050	ND		89.1246	ND	
	F52888-1	ND	3.0	0.1438	ND	ND	64.2262	ND	ND
	F52963-2	ND	3.0	0.1228	ND	ND	70.1610	ND	ND
	F52963-3	ND	3.0	0.1345	ND		70.1610	ND	

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.20	80%										
2	Drift	1.50	1.23	82%										
3	Wash		ND											
4	Std 1	0.015	0.016	104%										
5	Std 2	0.03	0.03	101%										
6	Std 3	0.06	0.06	97%										
7	Std 4	0.09	0.09	99%										
8	Std 5	0.12	0.13	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	98%										
11	Std 8	0.60	0.60	101%										
12	Std 9	1.20	1.23	102%										
13	Std 10	1.50	1.47	98%										
14	Drift	1.50	1.25	83%										
15	Wash		ND											
16	Blk-1		ND		3									
17	Blk-2		ND		3									
18	Spk-1		ND		3					0.004	63	0.15	ND	0%
19	Spk-2		ND		3					0.004	63	0.15	ND	0%
20	Spk-3		0.06		3					0.004	63	0.15	0.17	112%
21	F52900-1		ND		3	0.1474	ND	61.3555	ND					
22	F52900-2		ND		3	0.1153	ND	61.3555	ND					
23	F52900-3		ND		3	0.1150	ND	61.3555	ND					
24	F52968-1		ND		3	0.1448	ND	66.6415	ND					
25	F52968-2		ND		3	0.1159	ND	66.6415	ND					
26	Drift	1.50	1.26	84%										
27	Wash		ND				ND		ND					
28	F52968-3		ND		3	0.1211	ND	66.6415	ND					
29	F52884-1		ND		3	0.1463	ND	64.9889	ND					
30	F52884-2		ND		3	0.1011	ND	64.9889	ND					
31	F52884-3		ND		3	0.1345	ND	64.9889	ND					
32	F52892-2		ND		3	0.1342	ND	68.7067	ND					
33	F52548-2		ND		3		ND		ND					
34	F52888-1		ND		3	0.1438	ND	64.2262	ND					
35	F52963-2		ND		3	0.1228	ND	70.1610	ND					
36	F52963-3		ND		3	0.1345	ND	70.1610	ND					

AMBT 13195.1
HWI 6329-133
Guerro

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
37	Blk		ND		3									
38	Drift	1.50	1.26	84%										
39	Wash		ND											

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.21	81%										
2	Drift	1.50	1.23	82%										
3	Wash		ND											
4	Std 1	0.015	0.016	108%										
5	Std 2	0.03	0.03	98%										
6	Std 3	0.06	0..0578											
7	Std 4	0.09	0.09	100%										
8	Std 5	0.12	0.12	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	98%										
11	Std 8	0.60	0.60	101%										
12	Std 9	1.20	1.23	102%										
13	Std 10	1.50	1.47	98%										
14	Drift	1.50	1.25	83%										
15	Wash		ND											
16	Blk-1		0.07		3.0									
17	Blk-2		0.02		3.0									
18	Blk-3		0.02		3.0									
19	Spk-1		0.05		3.0					0.004	63.00	0.15	0.15	100%
20	Spk-2		0.05		3.0					0.004	63.00	0.15	0.16	103%
21	Spk-3		0.05		3.0					0.004	63.00	0.15	0.15	96%
22	F52891-1		ND		3.0	0.1140	ND	64.4869	ND					
23	F52891-2		ND		3.0	0.1443	ND	64.4869	ND					
24	F52891-3		ND		3.0	0.1284	ND	64.4869	ND					
25	F52892-1		0.02		3.0	0.1322	0.43	68.7067	29.31					
26	Drift	1.50	1.25	83%										
27	Wash		ND											
28	F52965-1		0.02		3.0	0.1246	0.40	68.6665	27.78					
29	F52965-3		0.02		3.0	0.1510	0.31	68.6665	21.15					
30	Drift		1.26											
31	Wash		ND											

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.25	83%										
2	Drift	1.50	1.28	85%										
3	Wash		0.02											
4	Std 1	0.015	0.018	123%										
5	Std 2	0.03	0.03	88%										
6	Std 3	0.06	0.06	100%										
7	Std 4	0.09	0.09	98%										
8	Std 5	0.12	0.12	104%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	96%										
11	Std 8	0.60	0.61	101%										
12	Std 9	1.20	1.24	104%										
13	Std 10	1.50	1.46	97%										
14	Drift	1.50	1.31	87%										
15	Wash		0.02											
16	Blk-1		0.02		3.0									
17	Blk-2		0.02		3.0									
18	Blk-3		ND		3.0									
19	Spk-1		0.06		3.0					0.004	63.00	0.15	0.17	113%
20	Spk-2		0.05		3.0					0.004	63.00	0.15	0.15	100%
21	Spk-3		0.05		3.0					0.004	63.00	0.15	0.16	107%
22	Spk-4		0.06		3.0					0.004	63.00	0.15	0.18	120%
23	Spk-5		0.06		3.0					0.004	63.00	0.15	0.19	124%
24	Spk-6		0.06		3.0					0.004	63.00	0.15	0.18	117%
25	F52874-1		0.02		3.0	0.1438	0.46	69.6893	32.13					
26	Drift	1.50	1.27	84%										
27	Wash		0.02											
28	F52874-2		0.02		3.0	0.1221	0.50	69.6893	34.93					
29	F52874-3		0.02		3.0	0.1454	0.35	69.6893	24.59					
30	F52879-1		0.02		3.0	0.1338	0.34	89.1246	30.17					
31	F52879-2		ND		3.0	0.1050	ND	89.1246	ND					
32	F52889-1		0.04		3.0	0.1139	1.17	68.6699	80.31					
33	F52889-2		0.05		3.0	0.1125	1.34	68.6699	91.74					
34	F52889-3		0.10		3.0	0.1410	2.18	68.6699	149.47					
35	F52894-1		0.05		3.0	0.1272	1.20	73.9834	88.64					
36	F52894-2		0.06		3.0	0.1183	1.43	73.9834	105.44					

ARDT 12195.1
HWI 6329-133
Jimeno

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
37	F52894-3		0.05		3.0	0.1185	1.17	73.9834	86.91					
38	Drift	1.50	1.28	85%										
39	Wash		0.02											
40	F52895-1		0.03		3.0	0.1513	0.62	58.0944	36.17					
41	F52895-2		0.03		3.0	0.1091	0.87	58.0944	50.64					
42	F52895-3		0.02		3.0	0.1537	0.44	58.0944	25.74					
43	Drift	1.50	1.31	87%										
44	Wash													

DDW 7/15/95
AMDT 13195.1
HWL 6329-133
Lewis

1995-03-28 17:30 **OutPut of : 950328F1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-28 15:28

Analysis File Name : C:\SKALAR\DATA\950328F1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

```
          Ö x - c1 ¢      x  = corrected value of the sample
          º ááááá º      c1 = corrected value of the concentration 1
Result = 10â   s   ì      s  = Slope of the electrode
```

a2 = -0.00000
a1 = 0.00092
a0 = -1.24810

Fluoride L
Calibration order = 2

Correlation : r = 0.99716

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00022
a0 = 0.00604

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1

User file : . TXT
Reproces : No

1995-03-28 17:30 OutPut of : 950328F1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
wt	iw	Initial Wash	1	1.000	3	0.056		0	219	65	
					4	0.0060		0	0	0	
1	t	Tracer	1	1.000	3	1.199		2065	2282	203	TV=1.2 ppm
					4	0.9756		2065	0	0	
2	d	Drift	1	1.000	3	1.230		2096	2312	380	TV=1.2 ppm
					4	0.9981		2096	0	0	
3	w	Wash	1	1.000	3	0.056		0	214	557	
					4	0.0060		0	0	0	
4	s1	Standard 1	1	1.000	3	0.062		43	258	732	
					4	0.0156		43	0	0	
5	s2	Standard 2	1	1.000	3	0.070		106	322	907	
					4	0.0303		106	0	0	
6	s3	Standard 3	1	1.000	3	0.088		214	432	1081	
					4	0.0579		214	0	0	
7	s4	Standard 4	1	1.000	3	0.109		325	544	1257	
					4	0.0893		325	0	0	
8	s5	Standard 5	1	1.000	3	0.135		440	661	1432	
					4	0.1250		440	0	0	
9	s6	Standard 6	1	1.000	3	0.152		506	728	1606	
					4	0.1469		506	0	0	
10	s7	Standard 7	1	1.000	3	0.293		895	1121	1781	
					4	0.2979		895	0	0	
11	s8	Standard 8	1	1.000	3	0.604		1410	1641	1956	
					4	0.5548		1410	0	0	
12	s9	Standard 9	1	1.000	3	1.229		2095	2332	2132	
					4	0.9974		2095	0	0	
13	s10	Standard 10	1	1.000	3	1.473		2335	2576	2305	
					4	1.1797		2335	0	0	
14	d	Drift	1	1.000	3	1.248		2113	2338	2482	TV=1.2 ppm
					4	1.0106		2113	0	0	
15	w	Wash	1	1.000	3	0.056		0	226	2661	
					4	0.0060		0	0	0	

Fluoride 1.5
Fluoride L

PPM
PPM

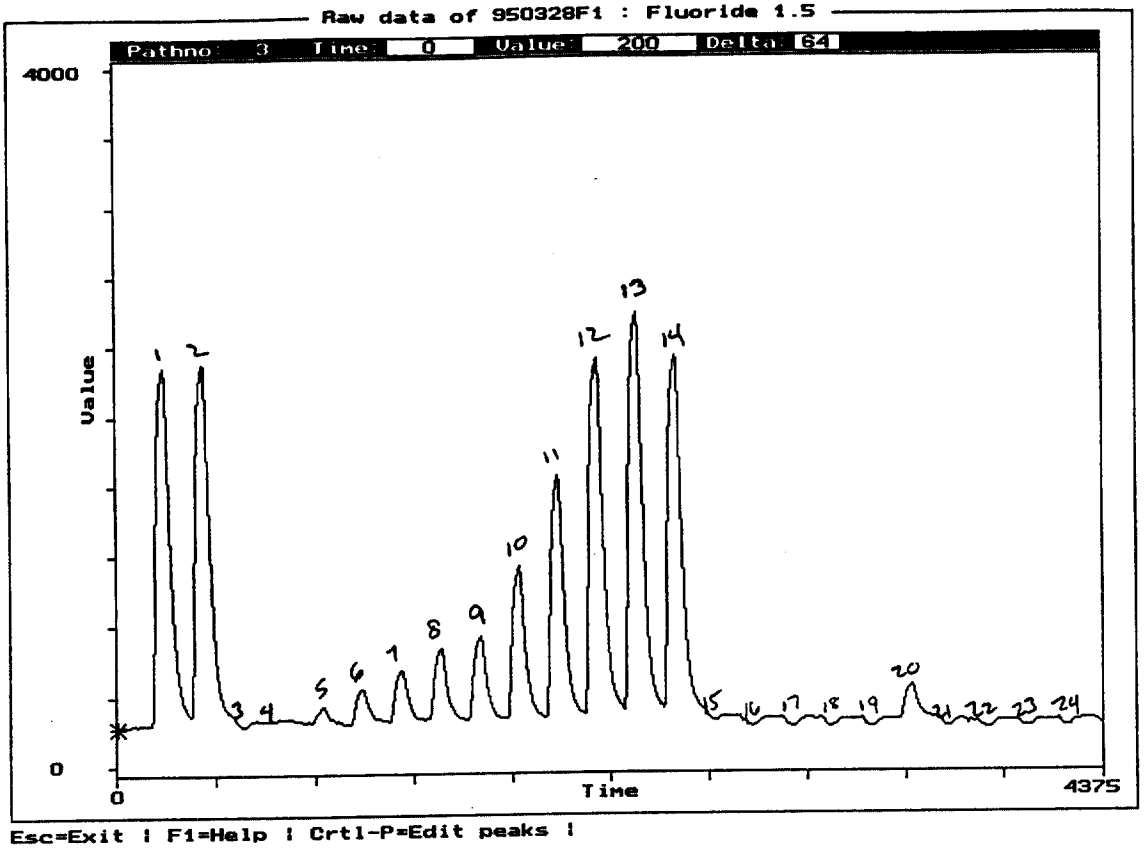
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
16	u	BLK 1	1	1.000	3	too e	<	14	240	2779	
					4	0.0091		14	0	0	ND
17	u	BLK 2	1	1.000	3	Absen	A	-32	194	3007	
					4	#.####		-32	0	0	ND
18	u	SPK 1	1	1.000	3	Absen	A	-23	202	3182	
					4	0.0011		-23	0	0	ND
19	u	SPK 2	1	1.000	3	Absen	A	-33	192	3357	
					4	#.####		-33	0	0	ND
20	u	SPK 3	1	1.000	3	0.087		209	436	3533	
					4	0.0566		209	0	0	0.1698
21	u	F52900-1	1	1.000	3	too l	>	17	242	3765	
					4	0.0098		17	0	0	ND
22	u	F52900-2	1	1.000	3	too e	<	-1	224	3820	
					4	0.0058		-1	0	0	ND
23	u	F52900-3	1	1.000	3	Absen	A	-33	192	4057	
					4	#.####		-33	0	0	ND
24	u	F52968-1	1	1.000	3	0.058		12	236	4184	
					4	0.0087		12	0	0	ND
25	u	F52968-2	1	1.000	3	Absen	A	-32	192	4407	
					4	#.####		-32	0	0	ND
26	d	Drift	1	1.000	3	1.259		2124	2348	4581	TV=1.2 ppm
					4	1.0187		2124	0	0	
27	w	Wash	1	1.000	3	0.056		0	224	4751	
					4	0.0060		0	0	0	
28	u	F52968-3	1	1.000	3	too e	<	32	256	4871	
					4	0.0131		32	0	0	ND
29	u	F52884-1	1	1.000	3	0.061		33	256	5089	.0399
					4	0.0133		33	0	0	ND .030
30	u	F52884-2	1	1.000	3	0.061		34	256	5282	.0405
					4	0.0135		34	0	0	ND .02
31	u	F52884-3	1	1.000	3	0.061		34	256	5435	.0405
					4	0.0135		34	0	0	ND .03

Dehrman Samples
Skalar @ 3x (not .045) 0.1 (.0

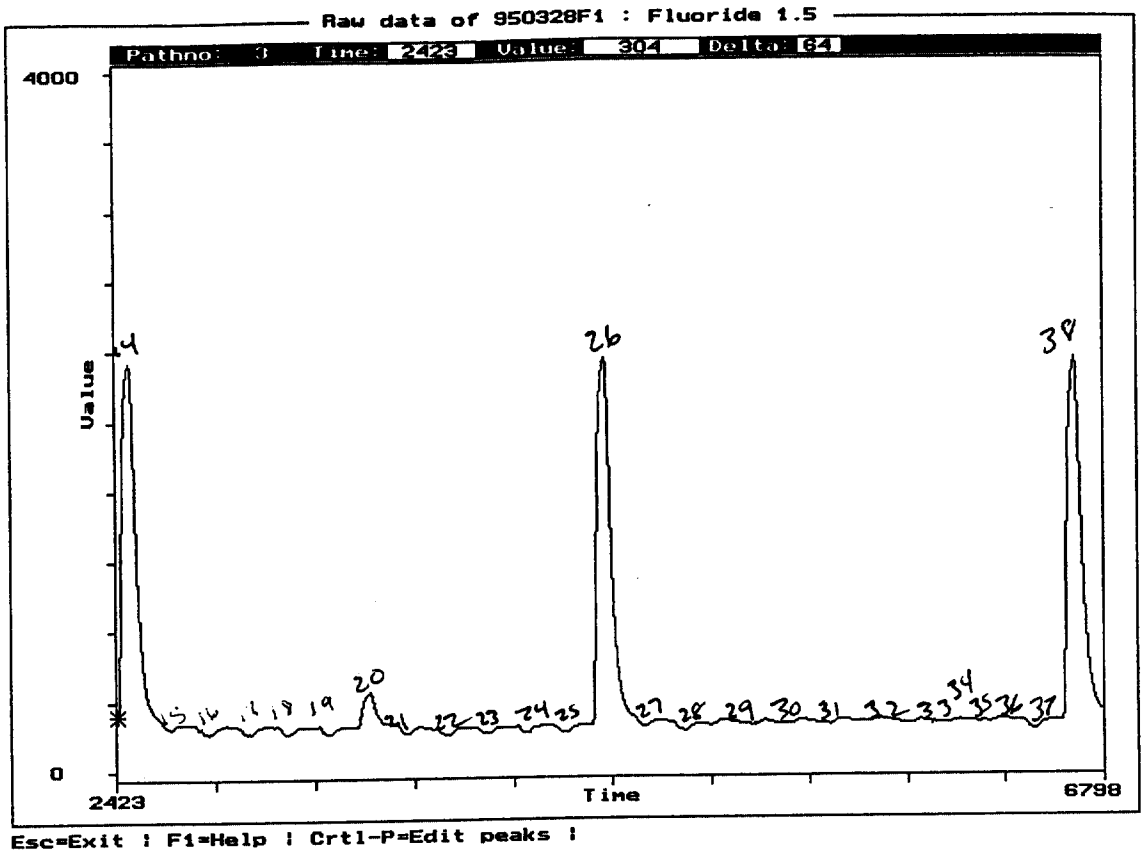
Fluoride 1.5
Fluoride L

PPM
PPM

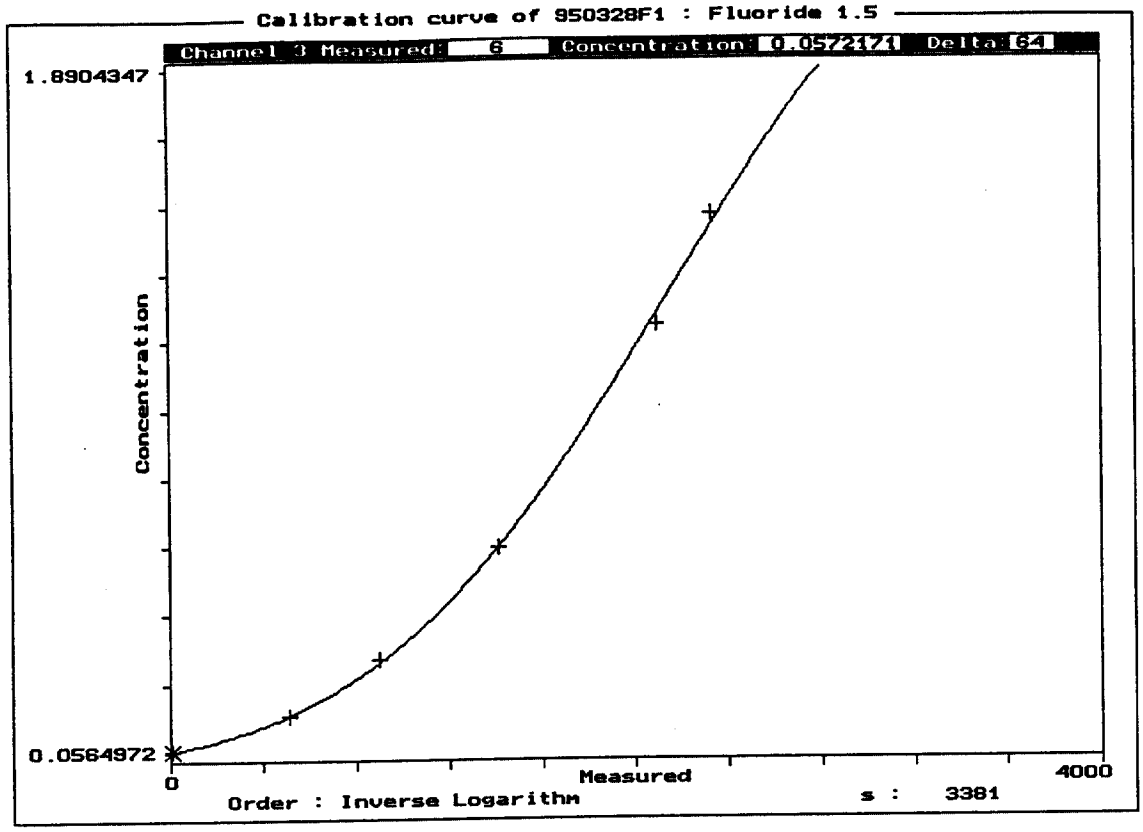
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	Dohrman	
32	u	F52892-2	1	1.000	3	0.061		38	260	5635	ND	035
					4	0.0144		38	0	0	.0432	N
33	u	F52548-2	1	1.000	3	0.061		39	260	5805	ND	
					4	0.0147		39	0	0		
34	u	F52888-1	1	1.000	3	0.061		36	256	5983	.0420	.022
					4	0.0140		36	0	0	ND	ND
35	u	F52963-2	1	1.000	3	0.061		36	256	6159	ND	.021
					4	0.0140		36	0	0	.042	N
36	u	F52963-3	1	1.000	3	0.061		36	256	6333	ND	.020
					4	0.0140		36	0	0	.042	N
37	u	~~F52963-4~~ Blank TISAB	1	1.000	3	Absen	A	-23	196	6506	ND	
					4	0.0011		-23	0	0		
38	d	Drift	1	1.000	3	1.257		2122	2340	6684	TV=1.2ppm	
					4	1.0172		2122	0	0		
39	w	Wash	1	1.000	3	0.056		0	218	6858		
					4	0.0060		0	0	0		
wt	rw	RunOut Wash	1	1.000	3	0.056		0	0	7159		
					4	0.0060		0	0	0		

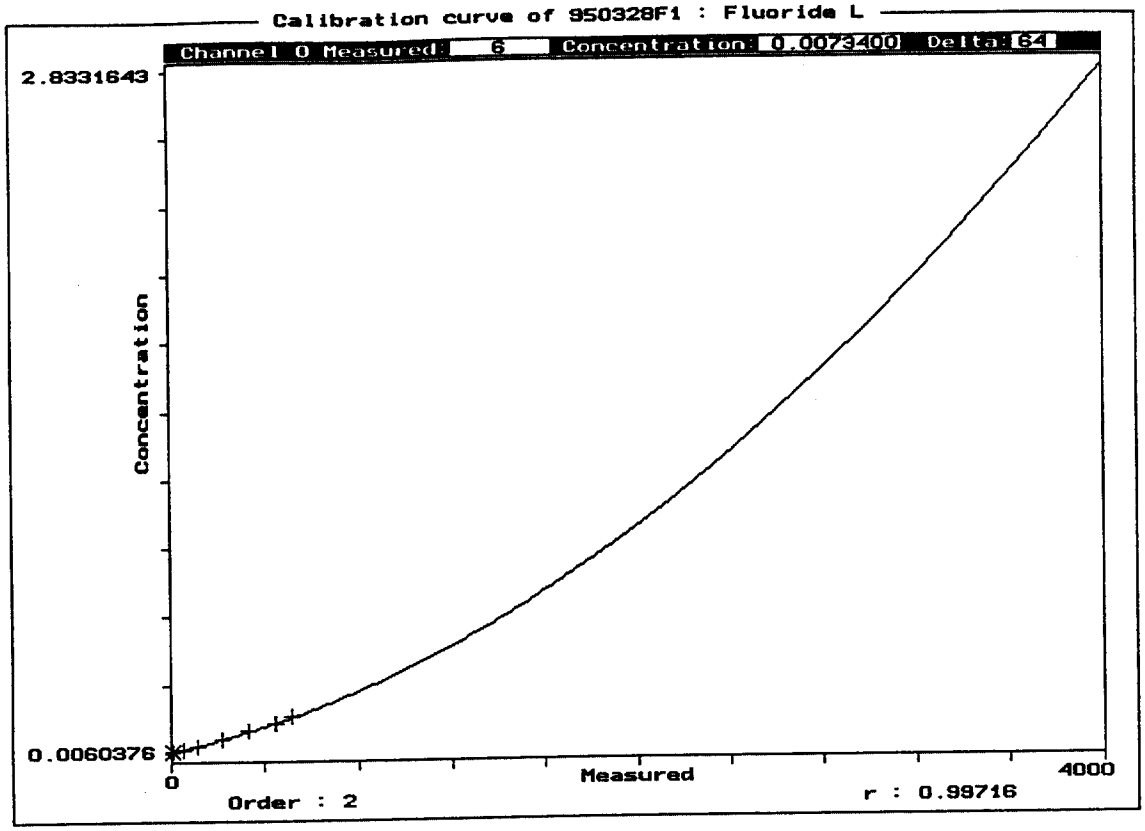


Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !



Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !


Calibration curve of 950328F1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0572171 Delta: 64
1.8904347
0.0564972
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 3381


Calibration curve of 950328F1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0073400
Delta: 64
2.8331643
0.0060376
Concentration
0
Measured
4000
Order : 2
r : 0.99716

DDW 7/18/95
AMDT 13195.1
HWI 6329-133
Lewis

1995-03-29 12:46 **OutPut of : 950329A1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-29 10:24

Analysis File Name : C:\SKALAR\DATA\950329A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

```
          Ö x - c1 ¢      x  = corrected value of the sample
          º ááááá º      c1 = corrected value of the concentration 1
Result = 10â   s    ì      s  = Slope of the electrode
```

a2 = -0.00000
a1 = 0.00087
a0 = -1.21638

Fluoride L
Calibration order = 2

Correlation : r = 0.99645

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00020
a0 = 0.00843

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

1995-03-29 12:46 OutPut of : 950329A1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

1995-03-29 12:46 OutPut of : 950329A1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
wt	iw	Initial Wash	1	1.000	3	0.061		0	216	65	
					4	0.0084		0	0	0	
1	t	Tracer	1	1.000	3	1.209		2068	2285	208	TV=1.2 ppm
					4	1.1307		2068	0	0	
2	d	Drift	1	1.000	3	1.234		2091	2308	382	
					4	1.1510		2091	0	0	
3	w	Wash	1	1.000	3	0.061		0	218	555	
					4	0.0084		0	0	0	
4	s1	Standard 1	1	1.000	3	0.065		37	258	732	
					4	0.0162		37	0	0	
5	s2	Standard 2	1	1.000	3	0.073		96	320	901	
					4	0.0294		96	0	0	
6	s3	Standard 3	1	1.000	3	0.091		208	436	1086	
					4	0.0578		208	0	0	
7	s4	Standard 4	1	1.000	3	0.112		320	552	1260	
					4	0.0902		320	0	0	
8	s5	Standard 5	1	1.000	3	0.136		425	660	1436	
					4	0.1244		425	0	0	
9	s6	Standard 6	1	1.000	3	0.152		489	728	1608	
					4	0.1470		489	0	0	
10	s7	Standard 7	1	1.000	3	0.293		893	1136	1784	
					4	0.3207		893	0	0	
11	s8	Standard 8	1	1.000	3	0.603		1415	1664	1957	
					4	0.6246		1415	0	0	
12	s9	Standard 9	1	1.000	3	1.228		2085	2342	2131	
					4	1.1457		2085	0	0	
13	s10	Standard 10	1	1.000	3	1.474		2308	2570	2309	
					4	1.3519		2308	0	0	
14	d	Drift	1	1.000	3	1.247		2103	2356	2483	TV=1.2 ppm
					4	1.1617		2103	0	0	
15	w	Wash	1	1.000	3	0.061		0	256	2663	
					4	0.0084		0	0	0	

Fluoride 1.5
Fluoride L

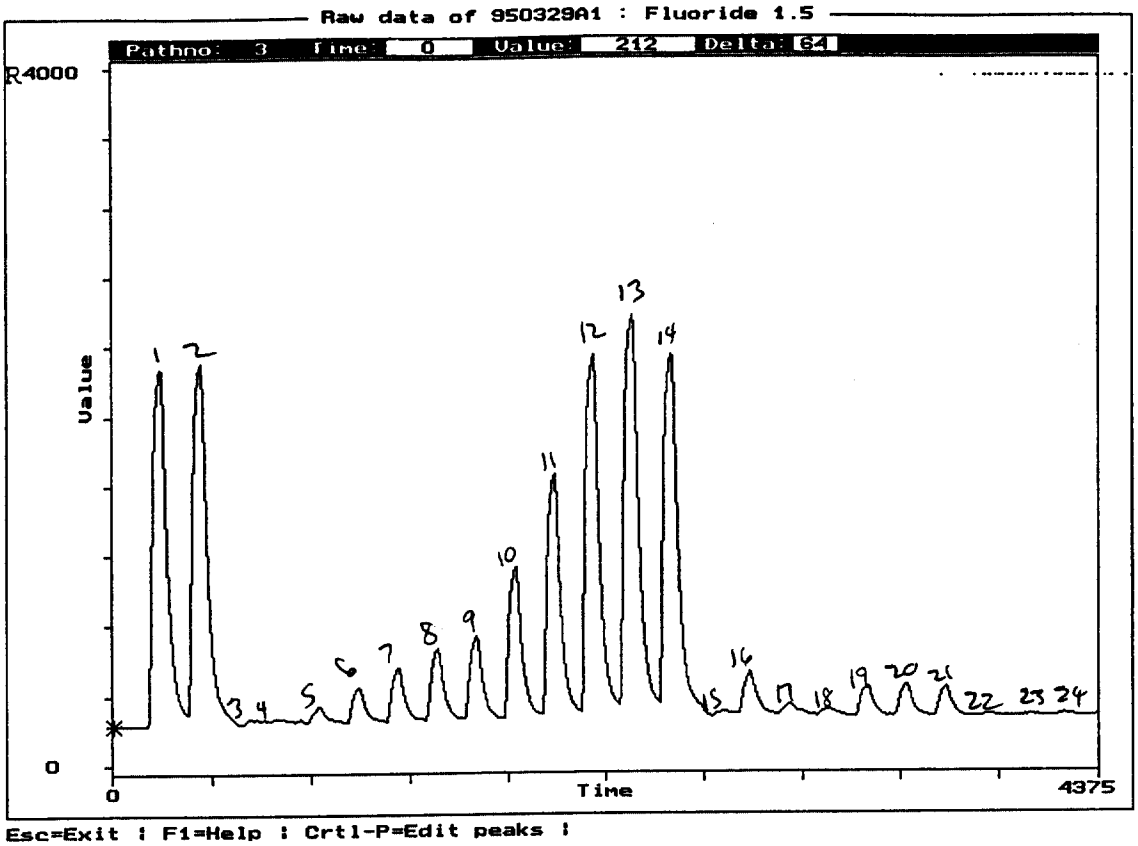
PPM
PPM

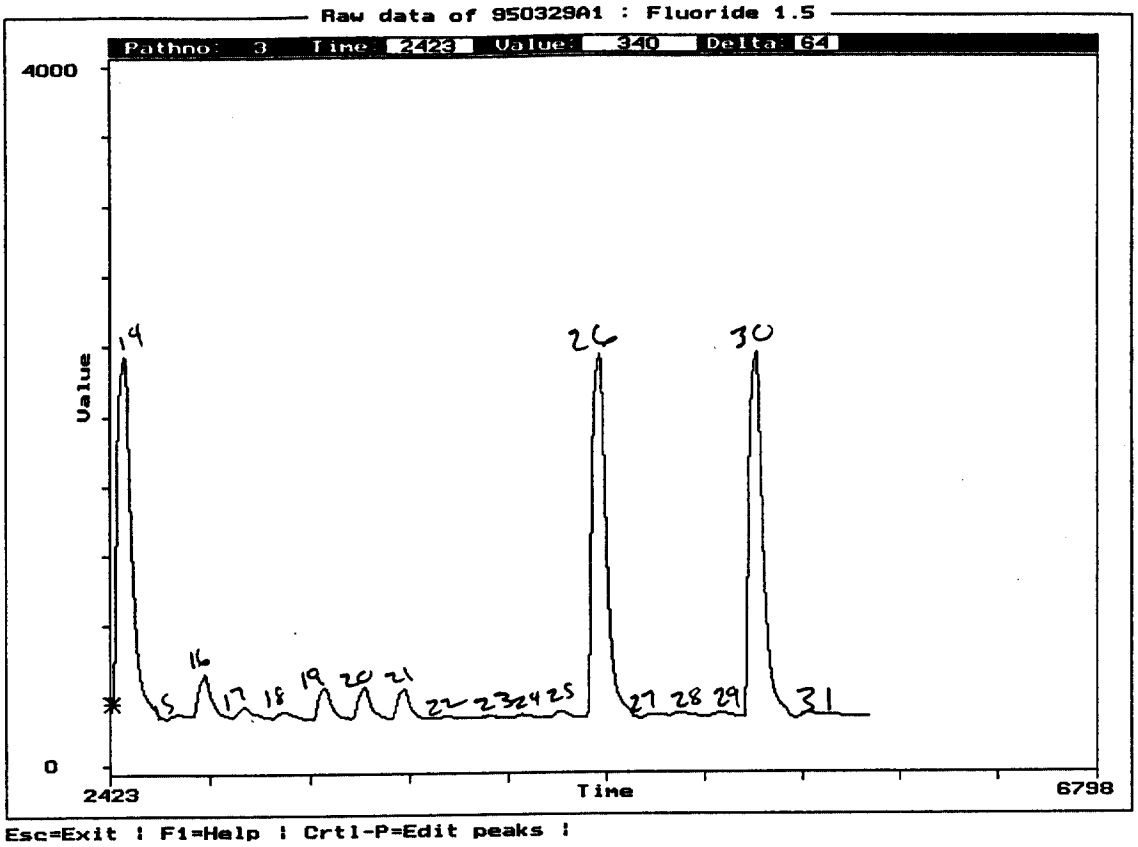
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time	
16	u	BLK 1	1	1.000	3	0.100		255	512	2829	
					4	0.0709		255	0	0	+20% .2127
17	u	BLK 2	1	1.000	3	0.070		68	321	3010	
					4	0.0230		68	0	0	+35% .069
18	u	BLK 3	1	1.000	3	0.067		48	300	3185	
					4	0.0186		48	0	0	.0558
19	u	SPK 1	1	1.000	3	0.086		180	432	3357	
					4	0.0503		180	0	0	+8% .1509
20	u	SPK 2	1	1.000	3	0.087		186	436	3533	
					4	0.0519		186	0	0	+15% .1557
21	u	SPK 3	1	1.000	3	0.085		173	422	3711	
					4	0.0485		173	0	0	+9% .1455
22	u	F52891-1	1	1.000	3	Absen	A	13	260	3883	
					4	0.0111		13	0	0	.0333 ⇒ ND
23	u	F52891-2	1	1.000	3	Absen	A	13	258	4058	
					4	0.0111		13	0	0	.0333 ⇒ ND
24	u	F52891-3	1	1.000	3	0.064		28	272	4231	
					4	0.0142		28	0	0	.0426 ⇒ ND
25	u	F52892-1	1	1.000	3	0.067		49	292	4410	
					4	0.0188		49	0	0	.0564
26	d	Drift	1	1.000	3	1.252		2107	2348	4584	TV=1.2 ppm
					4	1.1653		2107	0	0	
27	w	Wash	1	1.000	3	0.061		0	240	4747	
					4	0.0084		0	0	0	
28	u	F52965-1	1	1.000	3	0.066		40	280	4938	
					4	0.0168		40	0	0	.0504
29	u	F52965-3	1	1.000	3	0.065		34	274	5110	
					4	0.0155		34	0	0	.0465
30	d	Drift	1	1.000	3	1.263		2118	2358	5284	TV=1.2 ppm
					4	1.1752		2118	0	0	
31	w	Wash	1	1.000	3	0.061		0	240	5455	
					4	0.0084		0	0	0	

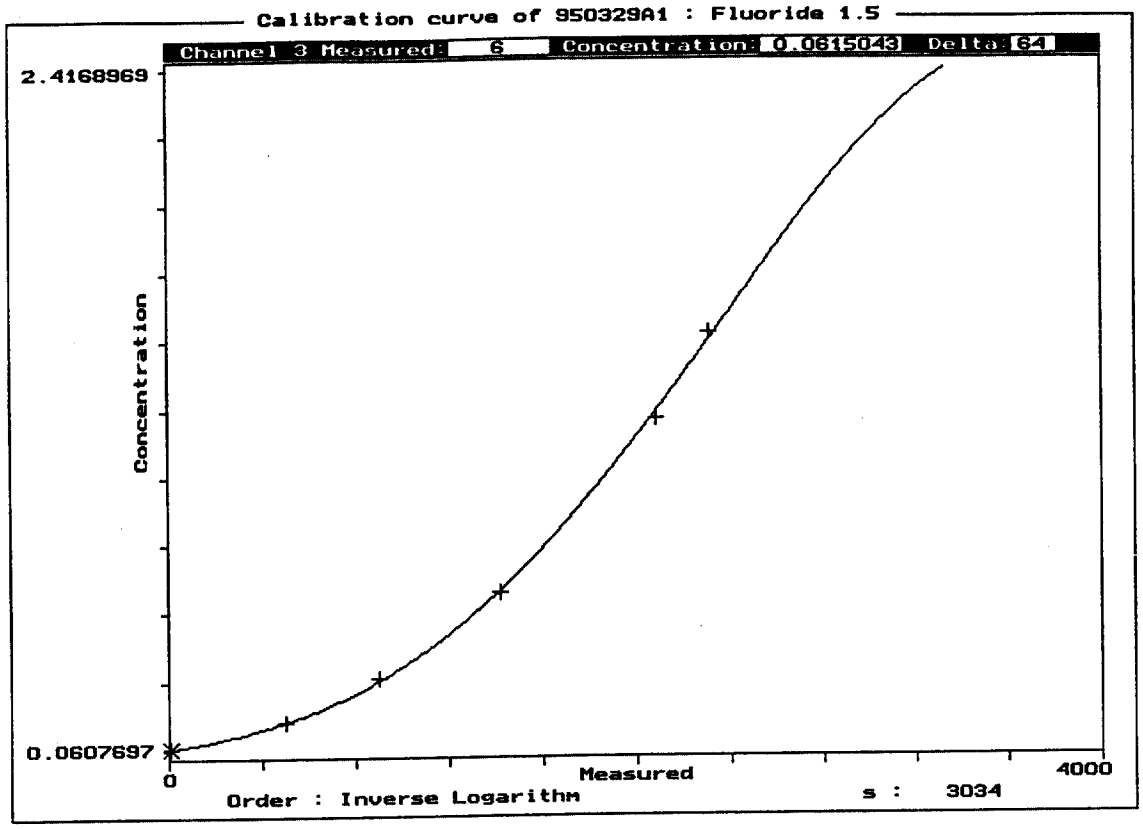
Fluoride 1.5
Fluoride L

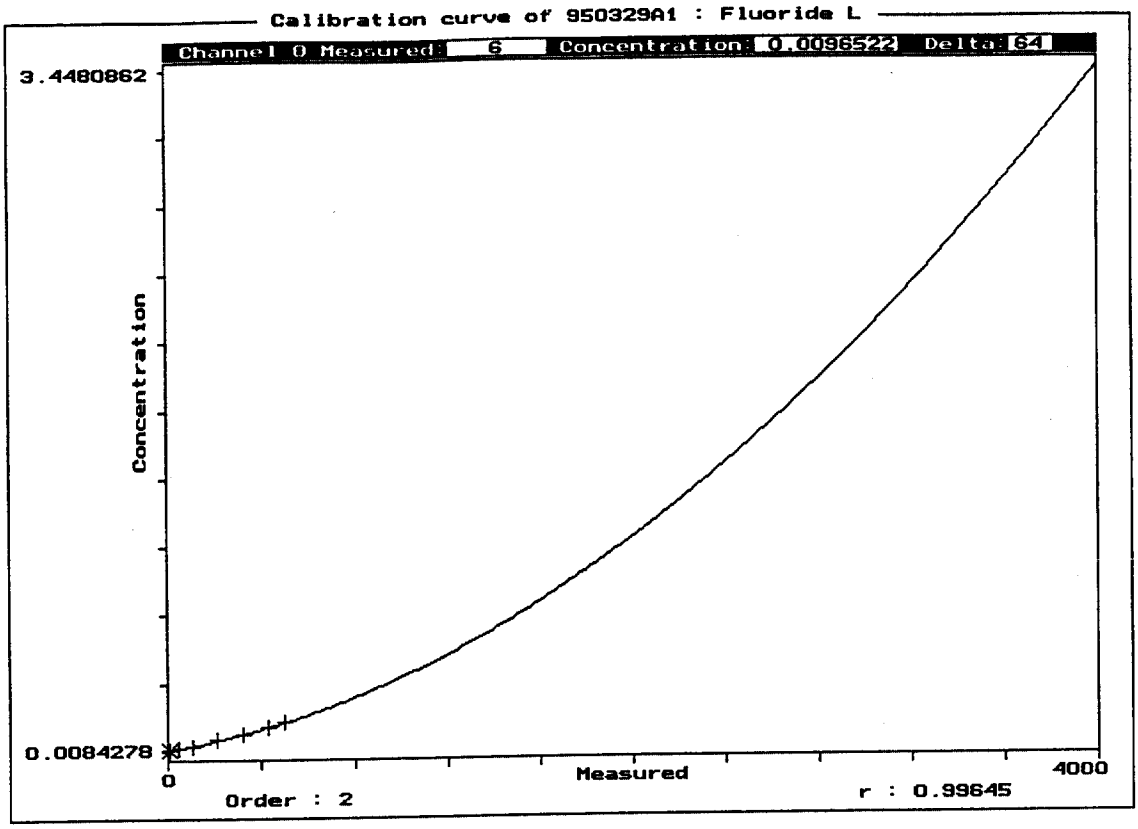
PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
wt	rw	RunOut Wash	1	1.000	3	0.061		0	256	5759
					4	0.0084		0	0	0


Raw data of 950329A1 : Fluoride 1.5
Pathno: 3 Time: 0 Value: 212 Delta: 64
R4000
Value
0
0
Time
4375
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !




Calibration curve of 950329A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0615043
Delta: 64
2.4168969
0.0607697
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 3034


Calibration curve of 950329A1 : Fluoride L
Channel 0 Measured: 6 Concentration: 0.0096522 Delta: 64
3.4480862
0.0084278
Concentration
0
4000
Measured
Order : 2
r : 0.99645

DDW 7/12/95
AMDT 13.9S.1
HWI 6329-133
Lucas

1995-03-30 10:59 **OutPut of : 950330A1**

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-03-30 08:43

Analysis File Name : C:\SKALAR\DATA\950330A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

Ö x - c1 ¢ x = corrected value of the sample
° ááááá ° c1 = corrected value of the concentration 1
Result = 10â s ì s = Slope of the electrode

a2 = -0.00000
a1 = 0.00088
a0 = -1.12348

Fluoride L
Calibration order = 2

Correlation : r = 0.99781

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00033
a0 = 0.01842

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file :	. TXT
	Reproces	: No

1995-03-30 10:59 OutPut of : 950330A1

Fluoride 1.5

```
Path number    :  3
Signal type    : Debubbled
Decolor        : Yes
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off

s1   sTandard  : Ignore
s2   sTandard  : Ignore
s3   sTandard  : Ignore
s4   sTandard  : Ignore
s5   sTandard  : Ignore
s6   sTandard  :    0.150
s7   sTandard  :    0.300
s8   sTandard  :    0.600
s9   sTandard  :    1.200
s10  sTandard  :    1.500
Order : Inverse Logarithm
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    :
output     : ##.###
```

Fluoride L

```
Path number    :  0
Signal type    : Debubbled
Decolor        : No
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off
```

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
wt	iw	Initial Wash	1	1.000	3	0.075		0	155	65
					4	0.0184		0	0	0
1	t	Tracer	1	1.000	3	1.248		2101	2274	211
					4	0.8362		2101	0	0
2	d	Drift	1	1.000	3	1.279		2139	2329	386
					4	0.8533		2139	0	0
3	w	Wash	1	1.000	3	0.075		0	208	569
					4	0.0184		0	0	0
4	s1	Standard 1	1	1.000	3	0.075		0	209	755
					4	0.0184		0	0	0
5	s2	Standard 2	1	1.000	3	0.079		24	234	906
					4	0.0264		24	0	0
6	s3	Standard 3	1	1.000	3	0.096		124	336	1084
					4	0.0599		124	0	0
7	s4	Standard 4	1	1.000	3	0.113		207	420	1262
					4	0.0882		207	0	0
8	s5	Standard 5	1	1.000	3	0.137		313	528	1435
					4	0.1248		313	0	0
9	s6	Standard 6	1	1.000	3	0.154		377	594	1612
					4	0.1472		377	0	0
10	s7	Standard 7	1	1.000	3	0.287		753	976	1785
					4	0.2835		753	0	0
11	s8	Standard 8	1	1.000	3	0.606		1308	1540	1962
					4	0.4990		1308	0	0
12	s9	Standard 9	1	1.000	3	1.242		2093	2338	2137
					4	0.8327		2093	0	0
13	s10	Standard 10	1	1.000	3	1.461		2383	2636	2312
					4	0.9645		2383	0	0
14	d	Drift	1	1.000	3	1.306		2173	2392	2487
					4	0.8686		2173	0	0
15	w	Wash	1	1.000	3	0.075		0	220	2729
					4	0.0184		0	0	0

Fluoride 1.5
Fluoride L

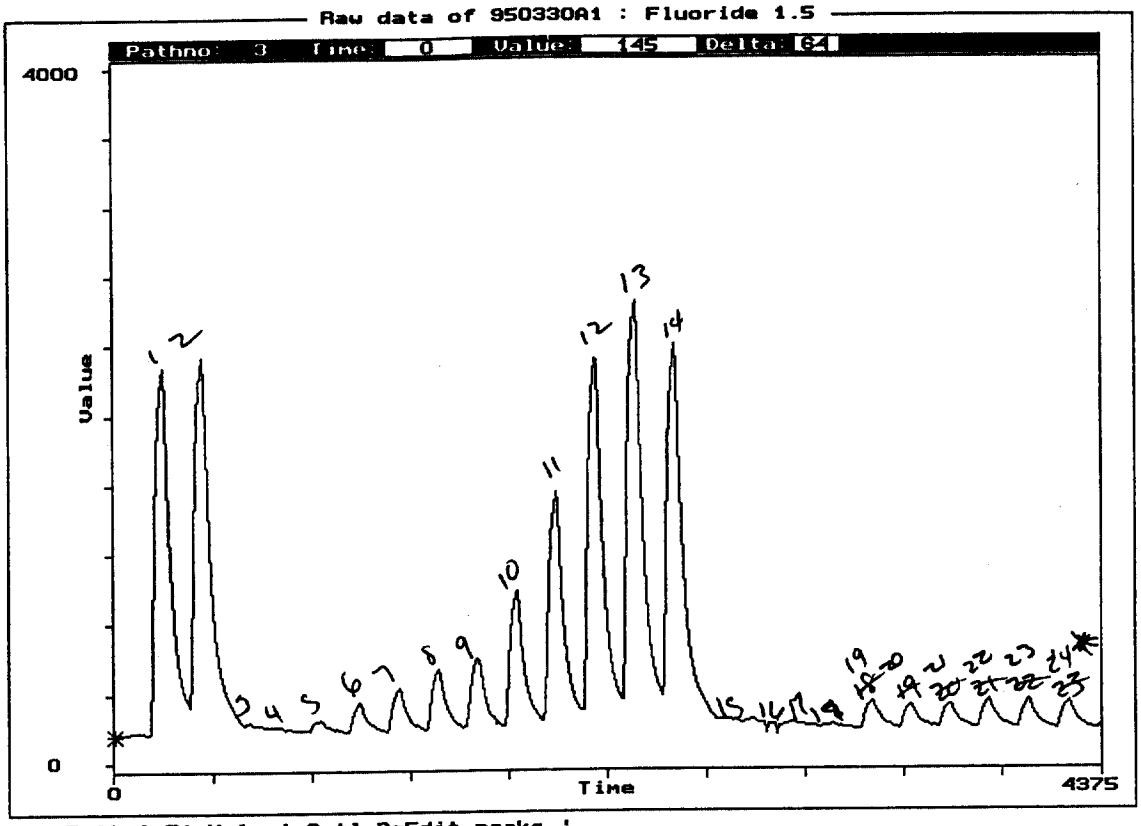
PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F Cor.	Valu	Time
16	u	blk 1	1	1.000	3	0.076	6	224	2822
					4	0.0204	6	0	0
17	u	blk 2	1	1.000	3	0.074	-7	208	3013
					4	0.0161	-7	0	0
18	u	blk 3	1	1.000	3	0.072	-21	192	3183
					4	0.0115	-21	0	0
19	u	spk 1	1	1.000	3	0.095	116	328	3363
					4	0.0572	116	0	0
20	u	spk 2	1	1.000	3	0.091	96	304	3536
					4	0.0505	96	0	0
21	u	spk 3	1	1.000	3	0.093	106	312	3706
					4	0.0539	106	0	0
22	u	spk 4	1	1.000	3	0.097	126	330	3888
					4	0.0606	126	0	0
23	u	spk 5	1	1.000	3	0.098	131	332	4063
					4	0.0623	131	0	0
24	u	spk 6	1	1.000	3	0.096	121	320	4235
					4	0.0589	121	0	0
25	u	F52874-1	1	1.000	3	0.077	11	208	4410
					4	0.0221	11	0	0
26	d	Drift	1	1.000	3	1.265	2122	2316	4587
					4	0.8456	2122	0	0
27	w	Wash	1	1.000	3	0.075	0	192	4766
					4	0.0184	0	0	0
28	u	F52874-2	1	1.000	3	0.076	6	196	4935
					4	0.0204	6	0	0
29	u	F52874-3	1	1.000	3	0.075	-4	184	5116
					4	0.0171	-4	0	0
30	u	F52879-1	1	1.000	3	0.074	-10	176	5274
					4	0.0151	-10	0	0
31	u	F52879-2	1	1.000	3	0.073	-16	169	5464
					4	0.0131	-16	0	0

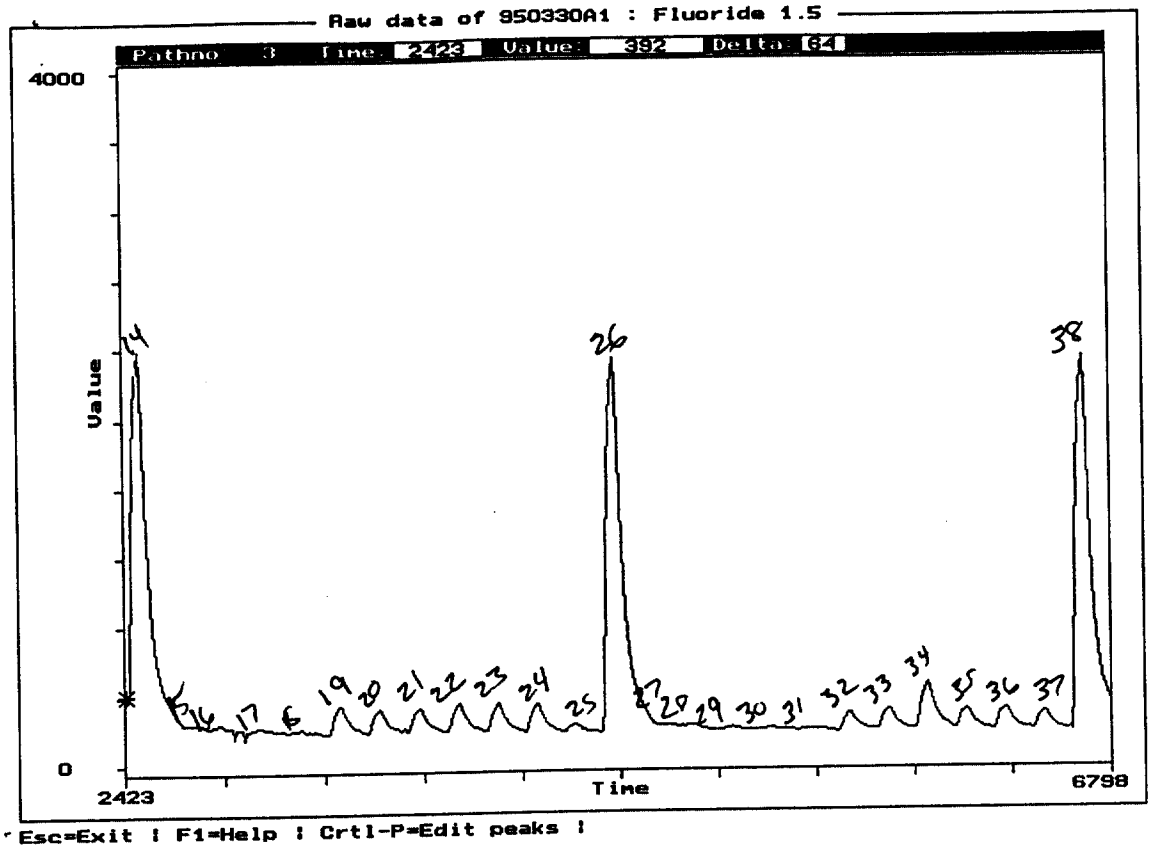
Fluoride 1.5
Fluoride L

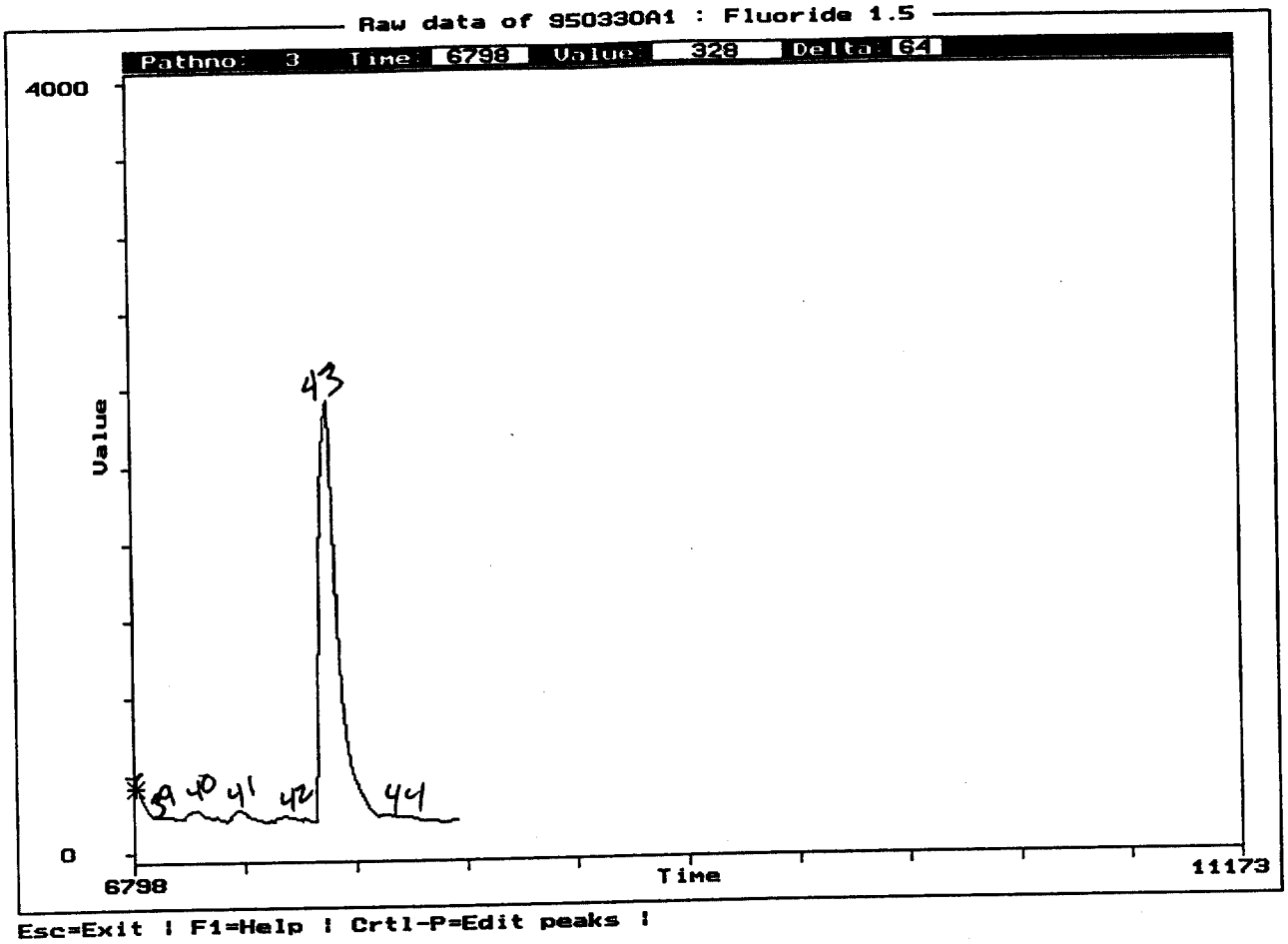
PPM
PPM

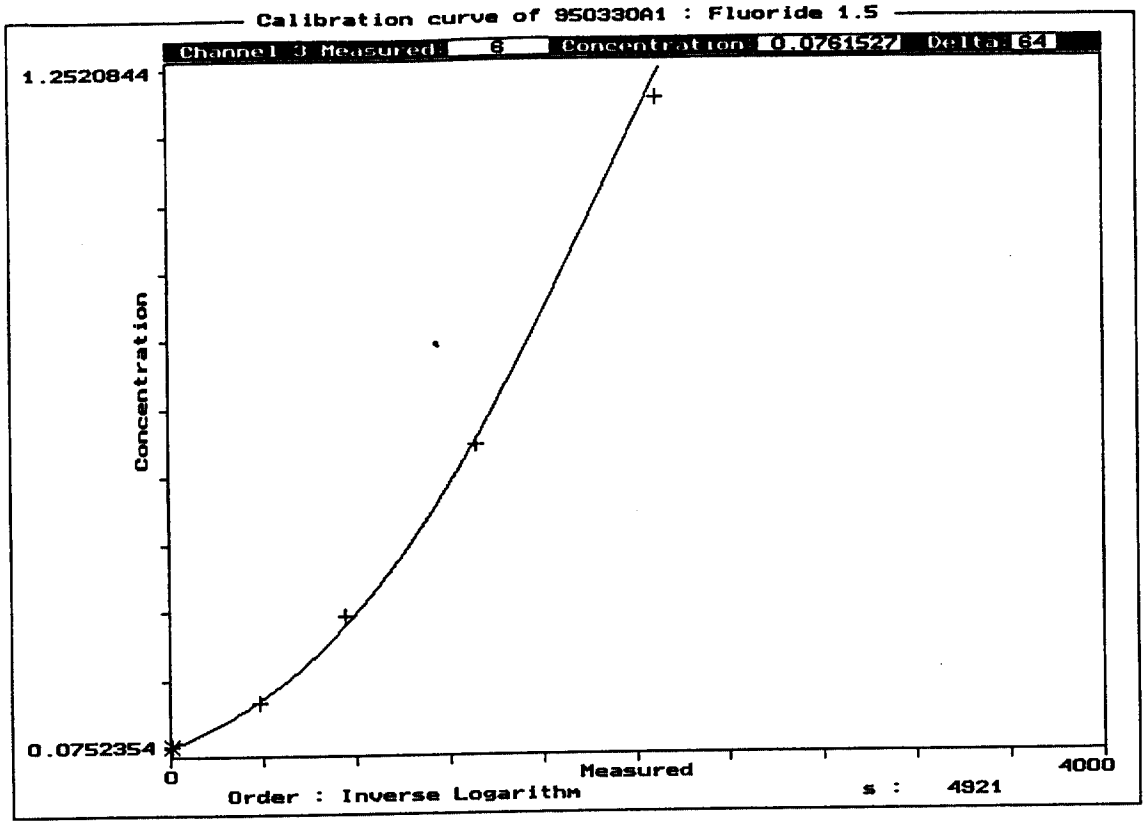
Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
32	u	F52889-1	1	1.000	3	0.088		78	261	5640
					4	0.0444		78	0	0
33	u	F52889-2	1	1.000	3	0.091		95	276	5812
					4	0.0501		95	0	0
34	u	F52889-3	1	1.000	3	0.122		248	426	5990
					4	0.1023		248	0	0
35	u	F52894-1	1	1.000	3	0.091		97	274	6164
					4	0.0508		97	0	0
36	u	F52894-2	1	1.000	3	0.094		113	288	6340
					4	0.0562		113	0	0
37	u	F52894-3	1	1.000	3	0.089		84	258	6514
					4	0.0464		84	0	0
38	d	Drift	1	1.000	3	1.276		2136	2308	6688
					4	0.8519		2136	0	0
39	w	Wash	1	1.000	3	0.075		0	170	6872
					4	0.0184		0	0	0
40	u	F52895-1	1	1.000	3	0.081		39	208	7034
					4	0.0314		39	0	0
41	u	F52895-2	1	1.000	3	0.082		40	208	7212
					4	0.0317		40	0	0
42	u	F52895-3	1	1.000	3	0.077		13	180	7388
					4	0.0227		13	0	0
43	d	Drift	1	1.000	3	1.310		2178	2344	7565
					4	0.8708		2178	0	0
44	w	Wash	1	1.000	3	0.075		0	165	7796
					4	0.0184		0	0	0
wt	rw	RunOut Wash	1	1.000	3	0.075		0	138	8040
					4	0.0184		0	0	0

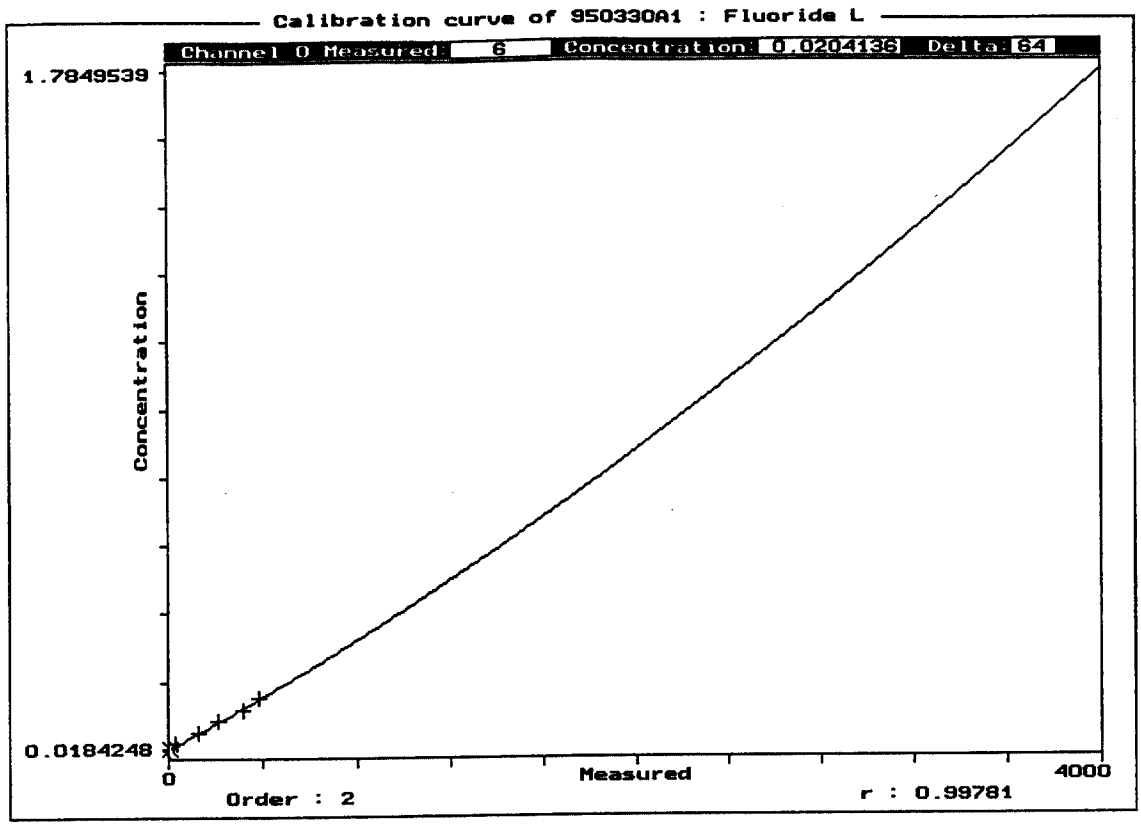


ADQWTE 7/18/95




Raw data of 950330A1 : Fluoride 1.5
Pathno: 3 Time: 6798 Value: 328 Delta: 64
4000
Value
0
43
39
40
41
42
44
6798
Time
11173
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !


Calibration curve of 950330A1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0761527 Delta: 64
1.2520844
0.0752354
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 4921


Calibration curve of 950330A1 : Fluoride L
Channel 0 Measured: 6 Concentration: 0.0204136 Delta: 64
1.7849539
0.0184248
Concentration
0
Measured
4000
Order : 2
r : 0.99781

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-138

Study Number: AMDT-122094.2
Test Substance: FC-129 (T-6054)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/22/95
Completion Date

1.0 SUMMARY

Liver samples at 48 hours post intravenous dose of FC-129 (T-6054) in rabbits were analyzed for total organic fluorine and perfluorooctanesulfonate.

After an intravenous dose of FC-129 in rabbits, there is a detectable increase of total organic fluorine in liver at 48 hours post dose at doses ranging from 0.128 to 12.8 mg/kg. A substantial amount of this total organic fluorine is in the form of perfluorooctanesulfonate. This pharmacokinetic study shows that there is a convenient marker (perfluorooctanesulfonate) for assessing the extent of dermal absorption.

2.0 INTRODUCTION

This study was performed in order to provide data for the assessment a subsequent dermal absorption study (HWI#6329-133). Knowing the disposition of an intravenous dose of FC-129, facilitates interpretation of a dermal absorption study.

Analysis of liver and serum samples from rabbits dosed intravenously with FC-129 for total organic fluoride (combustion analysis) and specific compounds (electrospray mass spectrometry) provide data as to whether fluorinated compounds are in the liver at 48 hours post dose and whether perfluorooctanesulfonate is present. Perfluorooctanesulfonate is a very good marker for a dermal study since previous work has shown it to be persistent in liver and serum in rabbits (biological half life >1 month).

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company.

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20±10° C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day absorption study, so all tissues will be retained.

4.0 EXPERIMENTAL - Overview

Serum and tissues from animals dosed as described (HWI#6329-138), were available for analysis for total organic fluorine and fluorinated compounds. The samples were analyzed by combustion and/or electrospray mass spectrometry to the extent necessary to provide sufficient data for the interpretation of a second study on the extent of dermal absorption of FC-129 (HWI#6329-133).

5.0 EXPERIMENTAL - METHODS

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

6.0 DATA ANALYSIS

The data are attached. For combustion analysis for total organic fluorine, the rabbits dosed with 0, 0.128, 0.64, 1.28, and 12.8 mg/kg had 18 (below the level of practical quantitation), 61, 118, 164, and 2239 ug/whole liver, respectively. The body weight

of the 12.8 mg/kg rabbit (F52752) was 2.7 kg. Thus, the total dose given this rabbit was 34.6 mg. The amount of total organic fluorine in this dose is 19 mg. The total organic fluorine in liver after the intravenous dose in rabbit F52752 represents 11.8% of the dose.

The data for an electrospray mass spectrometry analysis is attached. There is detectable perfluorooctanesulfonate in all treated rabbits. The high dose rabbit (F52752) had about 335 ug perfluorooctanesulfonate present in liver at 48 hours. If the 34.6 mg dose is expressed as FC-95, (that is, assuming 100% biotransformation) the dose is 31.8 mg. The 335 ug present in whole liver in this animal represents 1.05% of the dose. This represents a substantial amount of biotransformation of FC-129 to perfluorooctanesulfonate at 48 hours.

Other data was collected using Skalar segmented flow analyzer with ion selective electrode (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances that May Affect the Quality of the Data: The problem with this analysis is that in a pharmacokinetic study reported separately (HWI#6329-159), there was a delay in perfluorooctanesulfonate concentration observed in serum that indicated an unexplained distribution pattern. The level dropped dramatically at 12 and 24 hours but returned to a maximum at 48 hours with a subsequent decay in serum levels that indicated a biological half-life of > 1 month. This delay in perfluorooctanesulfonate levels may be shifted to a later time when perfluorooctanesulfonate is resulting from biotransformation. There may actually be a higher level of perfluorooctanesulfonate in liver at a later time period and this study only went to 48 hours. This would indicate that perfluorooctanesulfonate is an even better marker for a dermal absorption study than would be indicated by this data.

7.0 CONCLUSION

After an intravenous dose of FC-129 in rabbits, there is a detectable increase of total organic fluorine in liver at 48 hours at doses ranging from 0.64 to 12.8 mg/kg. A substantial amount of this total organic fluorine is in the form of perfluorooctanesulfonate. This pharmacokinetic study shows that there is a convenient marker for assessing the extent of dermal absorption.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-138 "Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-122094.2

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-122094.2

9.10 Instrument Settings: See methods

9.11 Data: See attached.

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.1.1 Protocol and Final Report: HWI#6329-138 "Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 24, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-138

Phone 608-241-4471
Fax 608-241-7227
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD. MADISON, WI 53704

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/09/94	12/09/94	Protocol Review	12/09/94	01/10/95
12/19/94	12/19/94	Animal Observation	12/19/94	01/10/95
02/02/95	02/02/95	Data/Report Review	02/02/95	03/10/95

Cecilia M. Danner
Representative, Quality Assurance Unit

2/24/95
Date

000424

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits

Test Material	T-6054
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable Experimental Start Date Experimental Termination Date	 December 17, 1994 December 19, 1994

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-6054 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle (sterile water for injection) or 0.128, 0.64, 1.28, or 12.8 mg of T-6054/kg of body weight (Groups 1 through 5, respectively). The dose volume was 0.5 mL/kg for all groups.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). A blood sample (approximately 4 mL) was collected from an auricular artery or marginal ear vein of the animals at 2-, 4-, 6-, 8-, 12-, and 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection), approximately 20 mL of blood was obtained from each animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. Approximately 48 hours post-injection, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and sent frozen to the Sponsor after termination of the in-life phase.

All five animals appeared normal throughout the study.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6054, when administered as a single intravenous injection to rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6054 and described as an amber liquid. The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The purity and stability of the USP grade control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. Any unused test material was returned to the Sponsor after completion of all testing according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any remaining vehicle may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, Michigan on November 16, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from the required temperature and humidity conditions existed, they were documented and considered to have had no adverse effect on the study outcome. Animal husbandry and housing at HWI complied with standards outlined in the "Guide for the Care and Use of Laboratory Animals".[1]

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,702 to 2,891 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg T-6054/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	*	0	0.5	1
2	T-6054	0.128	0.5	1
3	T-6054	0.64	0.5	1
4	T-6054	1.28	0.5	1
5	T-6054	12.8	0.5	1

* Sterile Water for Injection, USP.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Dose Preparation and Administration

The test material was diluted with Sterile Water for Injection to achieve a specific concentration for each dose level. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (approximately 30 to 60 seconds in duration). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collections

A blood sample (approximately 4 mL) was collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all animals at 2, 4, 6, 8, 12, and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection), approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were stored at room temperature until centrifuged and separated into serum and cellular fractions. The blood samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (approximately 48-hours post-injection), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not conducted, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C. After tissue/bile collection, the animals were discarded.

Shipment of Tissues

After completion of the in-life phase the blood samples, livers, bile, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106). The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual body weights at initiation are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. All five animals appeared normal throughout the study.

Pathology

All animals survived to termination of the experimental phase and were not examined grossly when sacrificed.

DISCUSSION

The level of systemic exposure of T-6054 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 0.128, 0.64, 1.28, and 12.8 mg/kg. All animals appeared normal throughout the study following administration of this material.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

2-24-95
Date

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Day 0
1	0	Female	F52792	2,784
2	0.128	Female	F52793	2,805
3	0.64	Female	F52750	2,891
4	1.28	Female	F52751	2,702
5	12.8	Female	F52752	2,783

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0	Female	F52792	Appeared normal	✓	✓	✓	✓	✓
2	0.128	Female	F52793	Appeared normal	✓	✓	✓	✓	✓
3	0.64	Female	F52750	Appeared normal	✓	✓	✓	✓	✓
4	1.28	Female	F52751	Appeared normal	✓	✓	✓	✓	✓
5	12.8	Female	F52752	Appeared normal	✓	✓	✓	✓	✓

✓ Indicates condition exists.

APPENDIX A

Protocol TP8084.PK

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707 7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6054 in Rabbits

Date:

December 13, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-138

Phone 608 241 4471 Fax 608 241 7227
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6054 in Rabbits

HWI No.	6329-138
Test Material	T-6054
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	December 17, 1994
Experimental Termination Date	December 19, 1994
Draft Report Date	January 23, 1995

1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure when the test material is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6054

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

D. Storage
Room temperature

E. Reserve Samples
Reserve samples will not be required for this study.

F. Retention
Any unused test material will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Sterile water for injection

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this USP grade material is considered to be adequate for the purposes of this study.

D. Storage
Refrigerated

E. Reserve Samples
See Section, 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.5 to 3.5 kg

(5) Number and Sex
5 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	0.128	1
3	0.64	1
4	1.28	1
5	12.8	1

a The dose volume will be 0.5 mL/kg of body weight.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.

(2) Reason for Dosing Route
Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration
Single dose

(4) Dose Preparation
The test material will be diluted with sterile water for injection to achieve a specific concentration for each dose level. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The prepared test mixtures will be stored at room temperature until administration.

D. Observation of Animals

(1) Clinical Observations
The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from either ear via the catheterization of the auricular artery or from the marginal ear vein at 2, 4, 6, 8, 12, and 24 hours post-injection. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal at the time of necropsy (48 hours post-injection). Approximately 20 mL of blood will be collected from moribund animals during the study, also, if possible. The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain a temperature of -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or his alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section E. Termination, (3) Sample Collection, will be collected.

(2) Scheduled Sacrifice
At approximately 48 hours post-injection, animals surviving to termination will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. An abbreviated gross necropsy examination will not be done, however, tissues will be collected.

(3) Sample Collection
The whole liver and bile from each animal dying during the study, sacrificed in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected. The tissues will be placed on dry ice immediately after collection and then placed in a freezer set to maintain a temperature of -20°C ±10°C.

The tissues (liver, bile, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.D.(3).(c). The Sponsor is responsible for the retention and disposition of the samples.

F. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects
Gross pathology findings (if applicable)
Gross pathology report (if applicable and requested by the Study Director)

9. Location of Raw Data, Records, and Final Report

Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Sample collection records
Shipping records
Pathology Records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

12-15-94
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

12-13-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12.13.94
Date

(6329-138.protdsk2)

9.1.2 Analytical protocol AMDT-122094.2

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits

In-Vivo Study Reference Number: HWI#6329-138

Study Number: AMDT-122094.2
Test Substance: FC-129 (T-6054)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25,1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson — Date
Study Director

John L. Butenhoff 10/30/95
John Butenhoff, PhD — Date
Sponsor Representative

1.0 PURPOSE

This study is performed in order to provide information necessary to assess the extent of dermal absorption of FC-129 (T-6054) in a subsequent dermal absorption study, HWI#6329-133.

The study is designed to provide information as to whether FC-129 and its metabolites are detectable in liver and other tissues, either as total organic fluorine or as specific compounds when the FC-129 is administered as an intravenous dose; and to ascertain whether perfluorooctanate will provide a marker for dermal absorption.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4-liver), Sigma Chemical Company (2.1.2, 2.1.4-serum)

2.3 Number of Test and Control Samples: Liver and serum from 4 test animals and 1 control animal, other biological tissues (kidney, bile, cellular fraction) will be available for analysis if deemed appropriate by the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues and serum from animals dosed as described (HWI#6329-138), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver. For confirmation of the presence of specific compounds in tissues and serum, electrospray mass spectrometry can be used.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include averages and standard deviations from different dose groups. If necessary, simple standard statistical tests such as the Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D. Johnson 10/3/95
Group Leader Date

Gale K. Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

James W. Johnson 10-31-95
Group Leader Date

Gale A. Von Buskirk 10-31-95
Quality Assurance Date

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 Reagents

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.

4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.

4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step **9.12** of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{171 \text{ mg liver}^{*} / 1 \text{ ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 Calibration

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 Storage Conditions for Standards

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in 8.3.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W	FC-95 approx 1 ppm actual ppm #W	FC-95 approx. 5 ppm actual ppm #W	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:	Date & Initials
Pipet 1 mL of Liver Solution	
Pipet 100 uL of 12 ppm Internal Standard	Std. #
Vortex 15 sec.	
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	
Pipet 5 mL of Ethyl Acetate	
Vortex 20-30 sec.	
Shake 20 min.	
Centrifuge 20-25 min.	
Remove a 4 mL aliquot of organic layer	
Blow down to near dryness (<0.25 mL) with N_2	
Add 1 mL of 1:1 Acetonitrile/H_2O	TN#
Vortex 15 sec.	
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.	

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 6/6/95
Date

Quality Assurance 6/6/95
Date

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES$^-$.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES$^-$, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 Using the Autosampler

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- 9.3.3.1 Push the sample button
- 9.3.3.2 Set sample loop size = 100 uL
- 9.3.3.3 Set inject/sample = 2
- 9.3.3.4 Set Cycle time = 0
- 9.3.3.5 Name the file: Livers
- 9.3.3.6 Identify the tray used
- 9.3.3.7 Add the samples to Queue by pressing "Enter"
- 9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

Revision Number: 0 **Revision Date:** None

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Von Benthuysen 9-27-95
Quality Assurance Date

Software: IBM MS Word, 6.0
Affected Documents: **AMDT-EP-26,** Operation and Maintenance of the Skalar Segmented Flow Analyzer

1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps

4.1.2 Autopipets, oxford or equivalent with plastic tips

4.1.3 Polypropylene volumetric flasks, 100 mL

4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.

4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent

4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.

4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.

4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.

4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.

4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Prepare calibration standards as in section 4.3.

8.2 Calibration

8.2.1 The standards are analyzed at the beginning of the run.

8.3 Storage Conditions for Standards

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 Start Up Procedure

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.
9.1.2 Put the fluoride electrodes in the electrode chamber.
9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.
9.1.4 Put the reagent-lines in the appropriate bottles.
9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**
9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 Starting a Run

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.
9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.
9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.
9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.
9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under **9.2.1** (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-M-1, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor | Original to: Study Director | Copies to: QAU Files

Study Title: **Single-dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits**

Study Number: AMDT-122094.2 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/14/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95

QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-122094.2, HWI 6329-138
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

	ug
Control Group	18.0
0.128 mg/kg dose (T6054)	60.5
0.64 mg/kg dose (T6054)	118
1.28 mg/kg dose (T6054)	164
12.8 mg/kg dose (T6054)	2239

FC129 PK ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	Liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (µg)	Dosage (mg/kg)
Liver Blank-1		1.11		0.145			
Liver Blank-2		0.371		0.137			
Liver Blank-3		0.253		0.128			
Liver Spike-1	97%	1.04		0.141			
Liver Spike-2	95%	0.860		0.168			
F52750-1		1.71		0.118			
F52750-2		1.53	1.59	0.121	73.9	118	0.64
F52750-3		1.54		0.130			
F52793-1		0.839		0.141			
F52793-2		0.840	0.879	0.125	68.8	60.5	0.128
F52793-3		0.959		0.123			
F52792-1		0.253		0.141			
F52792-2		0.234	0.225	0.129	80.2	18.0	0.0
F52792-3		0.187		0.148			
F52751-1		2.64		0.108			
F52751-2		2.34	2.45	0.133	66.8	164	1.28
F52751-3		2.37		0.133			
F52752-1		23.3		0.143			
F52752-2		24.5	23.6	0.142	94.9	2239	12.8
F52752-3		23.0		0.137			
Liver Blank-1		0.364		0.134			
Liver Blank-2		0.272		0.109			
Liver spike 63-1	97%	0.985		0.148			
Liver spike 63-2	96%	1.06		0.137			
Liver spike 126-1	95%	2.38		0.121			
Liver spike 126-2	82%	1.76		0.142			
Liver spike 126-3	83%	2.31		0.109			

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

A-1
Contains page
A-1 through A
11-2-95
DLC

Study:	Single-Dose Intravenous Pharmacokinetic
Protocol Number:	TP8084.PK
Test Material:	T-6054 in Rabbits (FC-129)
Matrix:	Liver
R Squared Value:	0.9826
Response Factor Amount:	1.92E00
Analyst:	DLC
Date:	4/6/95
Method:	AMDT-M-4
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE File:	040695A

Group Dose	Sample #	Ion Count Ratio *	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg
Group 1: 0 mg/kg	F52792	0.0245	1.2562	1	0.0299	80.1586	0.002
Group 2: 0.128 mg /kg	F52793	0.2346	1.3227	1	0.2722	68.8400	0.019
Group 3: 0.64 mg/kg	F52750	0.4983	1.2397	1	0.6169	73.9156	0.046
Group 4: 1.28 mg/kg	F52751	0.5016	1.2835	1	0.5998	66.7527	0.040
Group 5: 12.8 mg/kg	F52752	2.7225	1.1852	1	3.5254	94.8862	0.335

* Ratio of M+499 Ion Count/M+427 Ion Count

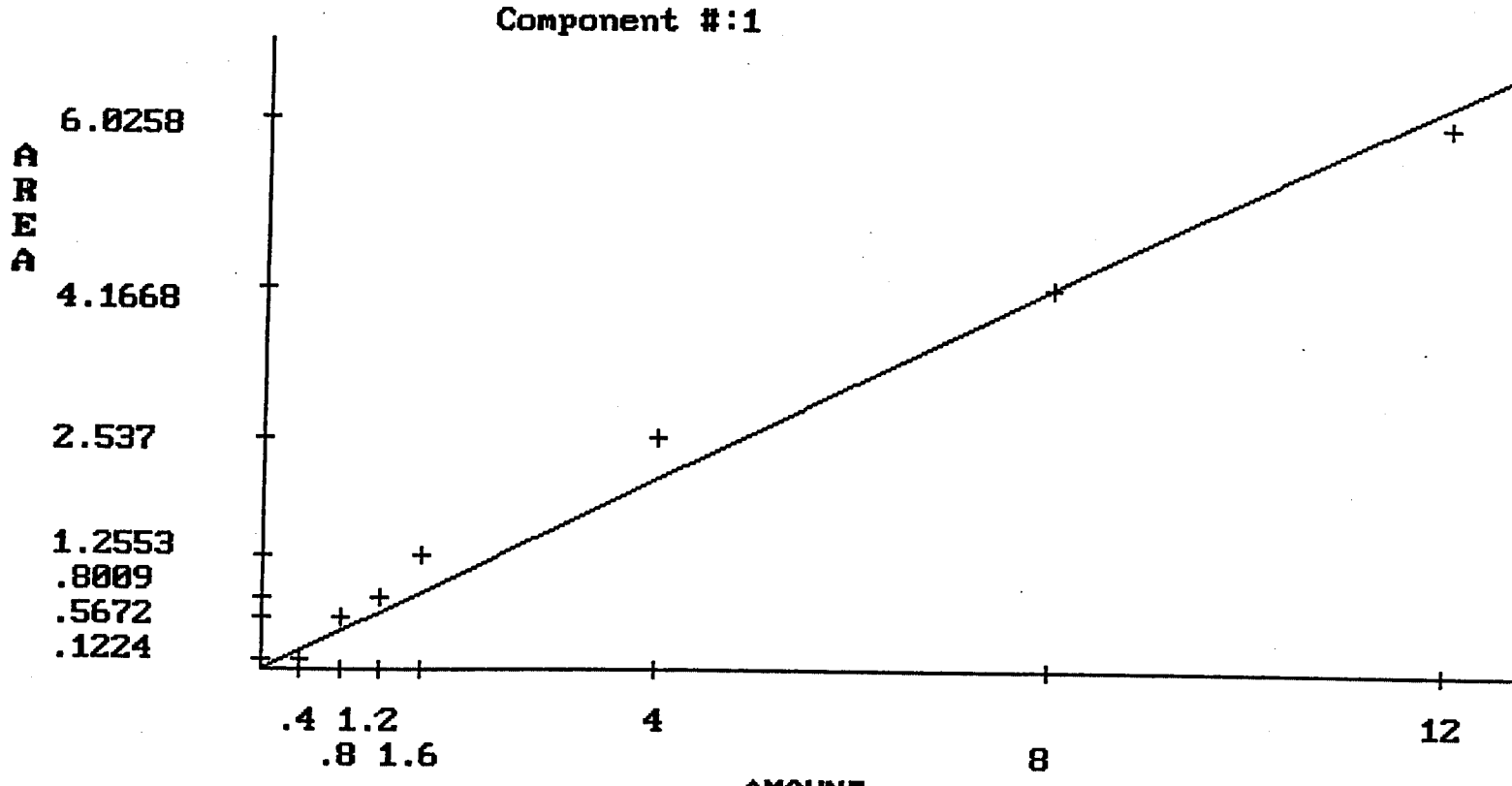
**The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

HWI# 6329-138

Conc.	499 Ion Count	427 Ion Count	M+499 I.C./M+427 I.C.
0.4	9091	74303	0.1224
0.8	63720	112333	0.5672
1.2	117051	146156	0.8009
1.6	183223	145956	1.2553
4	342495	135711	2.5237
8	654597	157098	4.1668
12	774077	128461	6.0258

Sample #	499 Ion Count	427 Ion Count	M+499 I.C./M+427 I.C.
F52792	2399	97837	0.0245
F52793	12826	54664	0.2346
F52750	41969	84226	0.4983
F52751	41908	83555	0.5016
F52752	256267	94129	2.7225

Method C:DLCLIVE1
Sample
Operator
Run date 07-26-1995 16:12:19 version: 35
Printed on 07-26-1995 AT 16:12:32
Straight Line Fit forced through Origin.



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	0
2	0.8000	1
3	1.2000	1
4	1.6000	1
5	4.0000	3
6	8.0000	4
7	12.0000	6

ratio

→ Area Ratio N⁻ 499 Area / N⁻ 427 Area
10/18/95 DLC

Y = SLOPE * X + INTERCEPT

Area = 5.2126E-01 * Amount + 0.0000E+00
Amount = 1.9184E+00 * Area + 0.0000E+00
R squared = 0.9826

Thurs 6 April 95

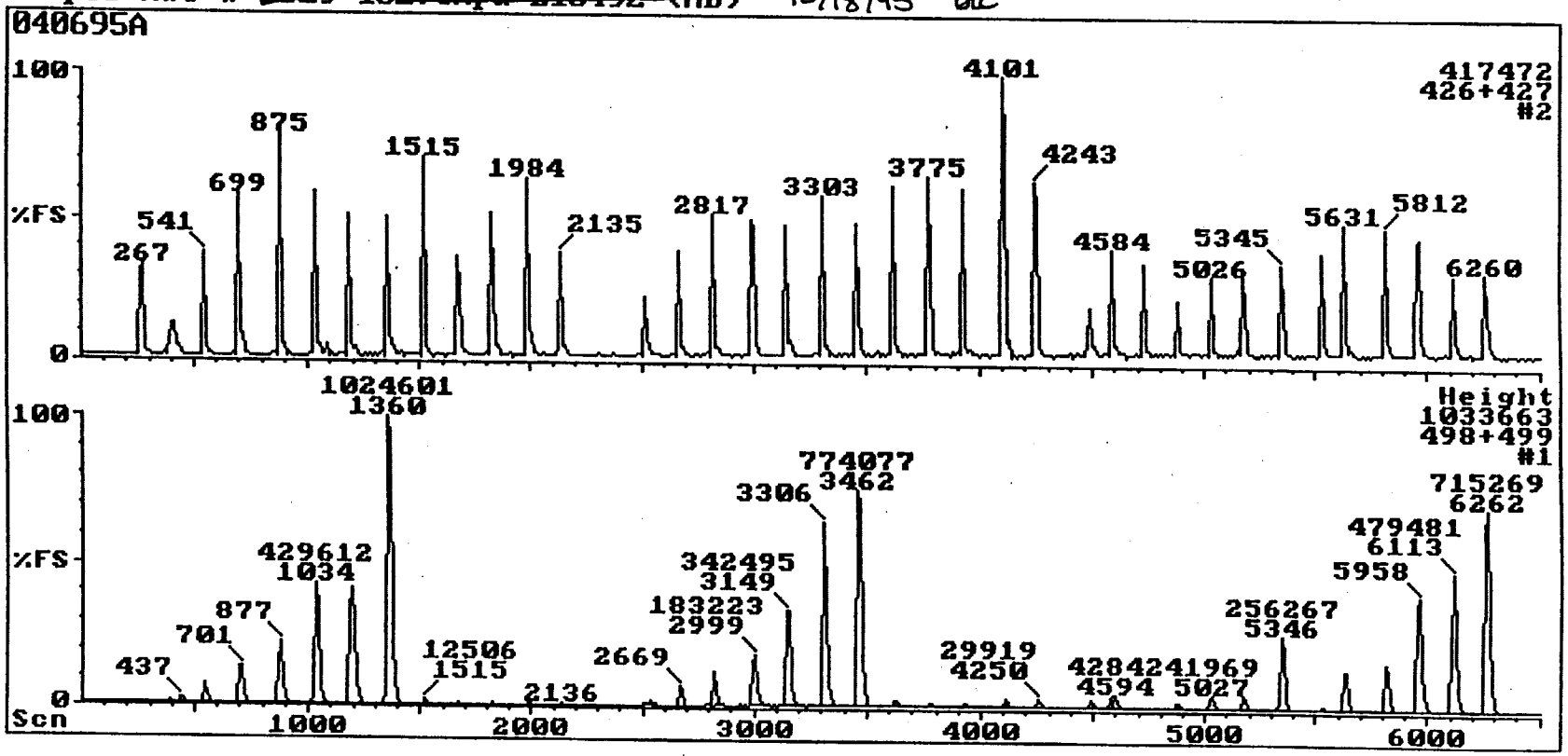
HWI# 6329-131 (FC-135)

- 136 (FC-99)

6329-138 (FC-129)

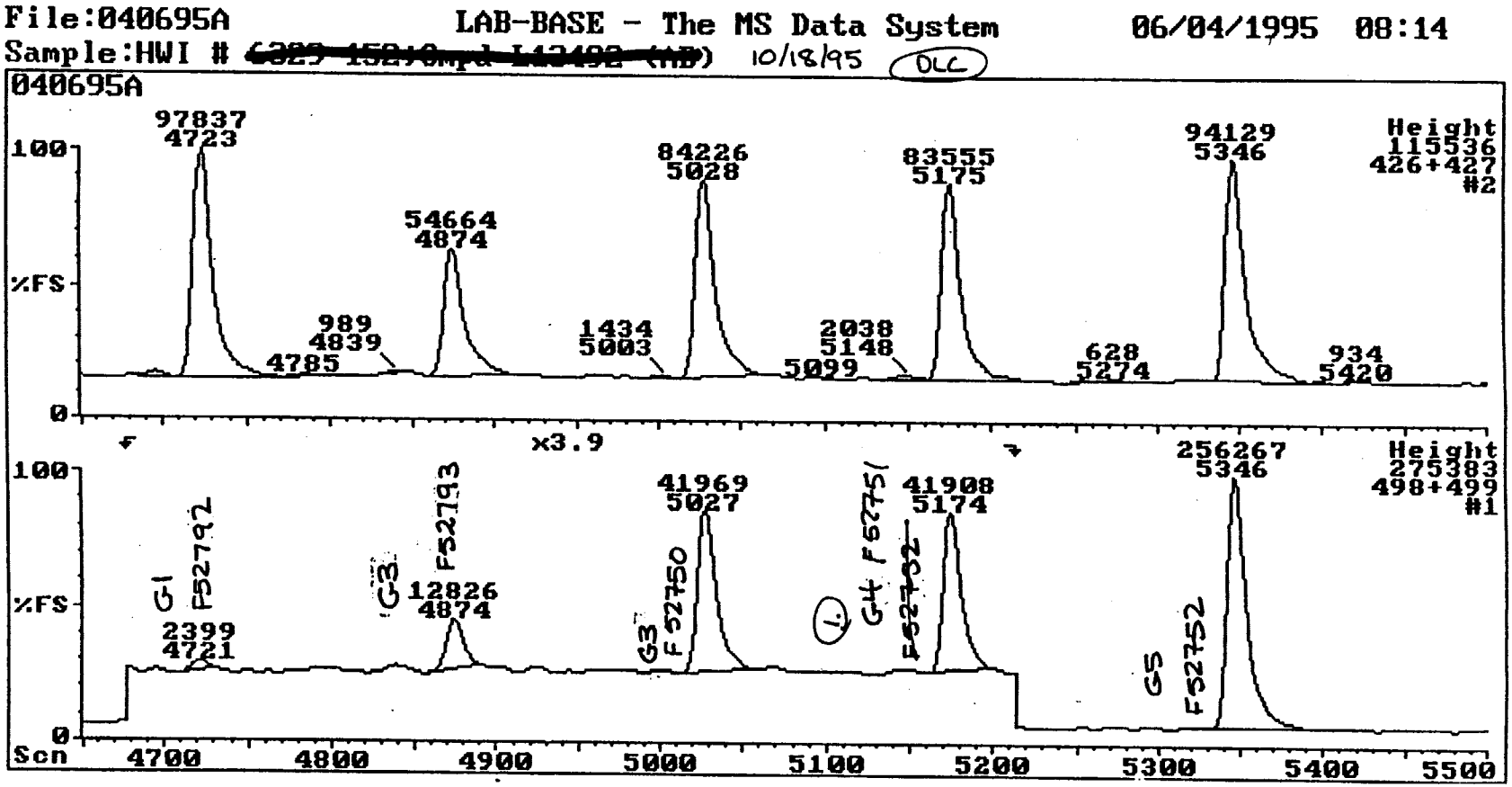
File:040695A LAB-BASE - The MS Data System 06/04/1995 08:14

Sample:HWI # ~~6329-152;Cmpd 140192 (MB)~~ 10/18/95 DLC



A-5

HWI #
6329-138 (FC-129)

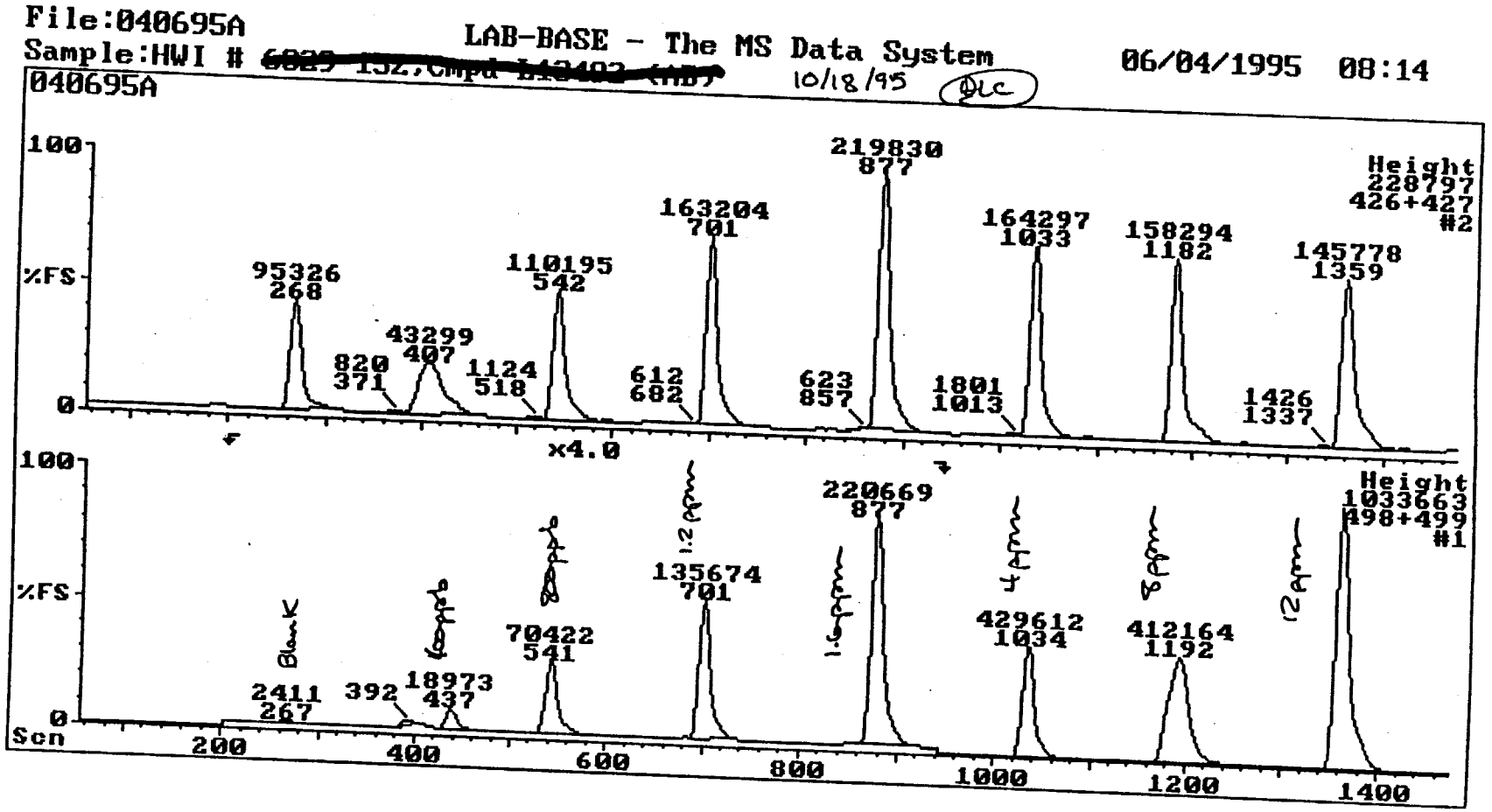


(1.) T.E. DLC 7/31/95
See Electrospray Log Book

G00492

A-6

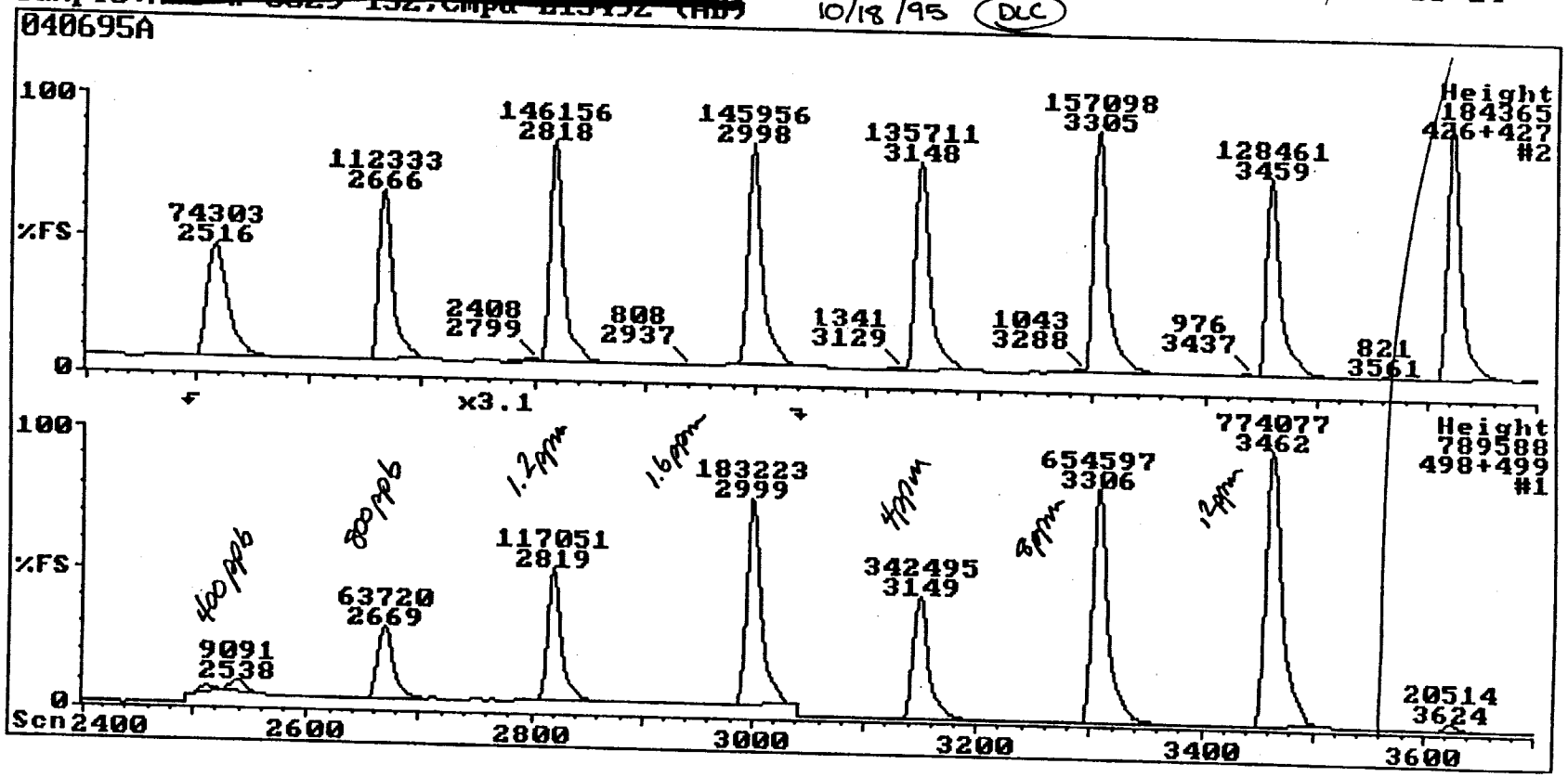
Beef Liver Standards 10/18/95 DLC



A-1

Beef Liver Standards 10/18/95 DLC

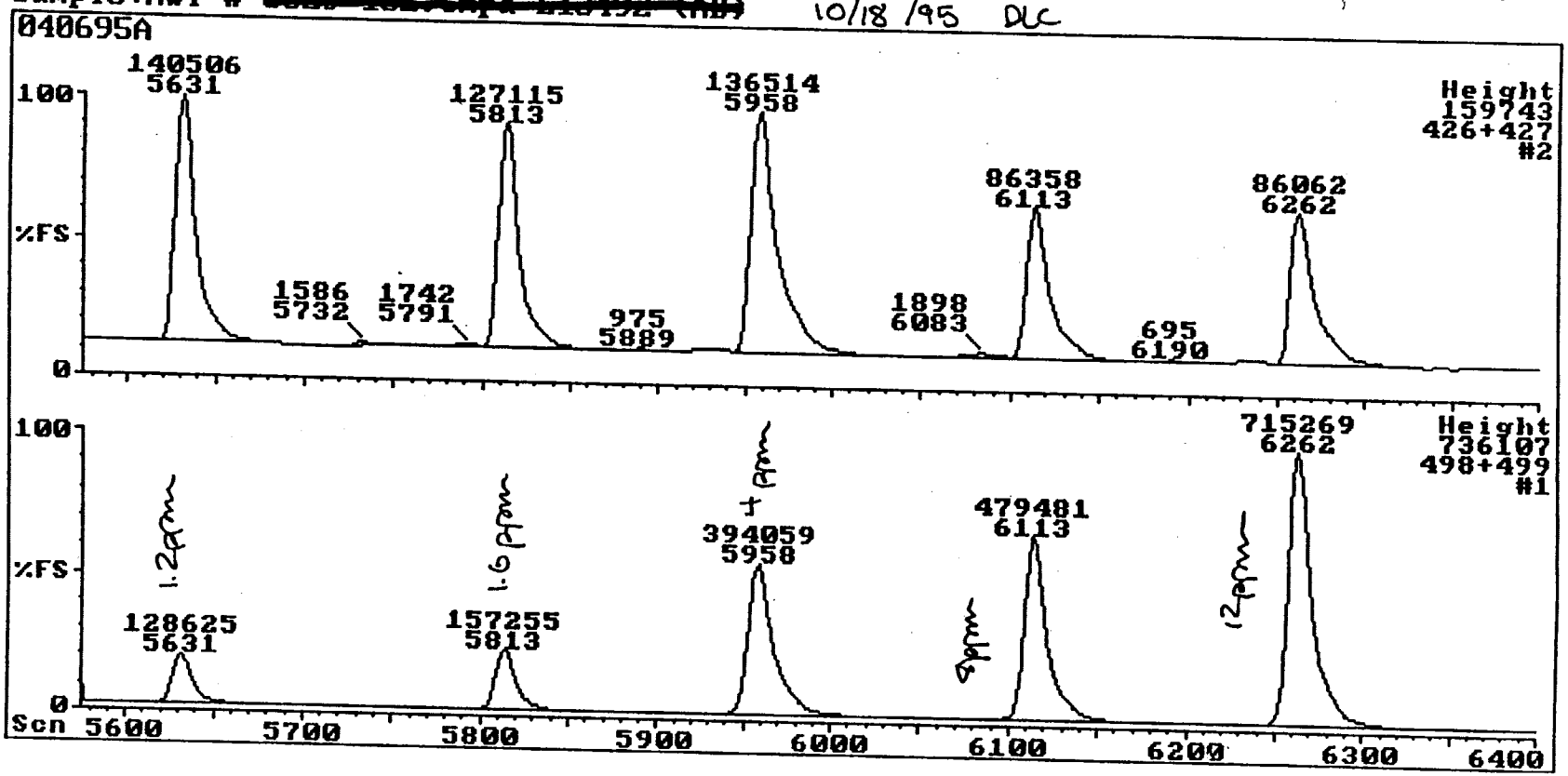
File:040695A LAB-BASE - The MS Data System 06/04/1995 08:14
Sample:~~HWI # 6329-152;Cmpd L13432 (AB)~~ 10/18/95 DLC



600494

A-8

File:040695A LAB-BASE - The MS Data System 06/04/1995 08:14
Sample:HWI # ~~6338-152;Cmpd L13492 (NB)~~ 10/18/95 DLC



000495

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

RE: 6329-138 LIVER SAMPLES
AMDT 122094.2
Date of Analysis: 4-20-95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using between 0.1 and 0.2 grams of the liver. The gas is collected in 1.0 mL of 1:1 TISAB/Milli-Q water then an additional 1 mL of 1:1 TISAB/Milli-Q is added to allow for sufficient volume for Skalar analysis. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Debra D Wright

SUMMARY OF 6329-138
LIVER SAMPLES
AMDT 122094.2

	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Sample Weight (grams)	Actual ppm F- in Sample	Average Actual ppm F- in Sample	Total Tissue Wt (grams)	Total F- per tissue (ug)	Average Total F- per tissue (ug)
GROUP 1	F52792-1	0.02	2.0	0.1409	0.27		80.1586	22	
Dose Level : 0	F52792-2	ND	2.0	0.1287	ND	ND	80.1586	ND	ND
	F52792-3	ND	2.0	0.1481	ND		80.1586	ND	
GROUP 2	F52793-1	0.07	2.0	0.1410	1.00		68.8400	69	
Dose Level : 0.128 mg/kg	F52793-2	0.06	2.0	0.1253	0.99	1.00	68.8400	68	69
	F52793-3	0.06	2.0	0.1229	1.01		68.8400	70	
GROUP 3	F52750-1	0.11	2.0	0.1176	1.95		73.9156	144	
Dose Level :0.64 mg/kg	F52750-2	0.11	2.0	0.1206	1.85	1.84	73.9156	137	136
	F52750-3	0.11	2.0	0.1303	1.73		73.9156	128	
GROUP 4	F52751-1	0.16	2.0	0.1078	3.02		66.7527	202	
Dose Level : 1.28 mg/kg	F52751-2	0.17	2.0	0.1332	2.58	2.74	66.7527	172	183
	F52751-3	0.17	2.0	0.1328	2.62		66.7527	175	
GROUP 5	F52752-1	0.42	2.0	0.1429	5.91		94.8862	560	
Dose Level : 12.8 mg/kg	F52752-2	0.76	2.0	0.1419	10.67	9.05	94.8862	1012	859
	F52752-3	0.73	2.0	0.1370	10.58		94.8862	1004	

1995-07-06 15:57 OutPut of : 950420A1
Operator : DDW
Date of the Analysis : 1995-04-20 07:12
Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950420A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.46	97%										
2	Drift	1.50	1.47	98%										
3	Wash		ND											
4	Standard 1	0.015	0.015	98%										
5	Standard 2	0.03	0.03	101%										
6	Standard 3	0.06	0.06	101%										
7	Standard 4	0.09	0.09	100%										
8	Standard 5	0.12	0.12	99%										
9	Standard 6	0.15	0.15	100%										
10	Standard 7	0.30	0.28	94%										
11	Standard 8	0.60	0.61	102%										
12	Standard 9	1.20	1.23	103%										
13	Standard 10	1.50	1.47	98%										
14	Drift	1.50	1.48	99%										
15	Wash		ND											
16	BLK-1		0.10		2.0	0.1445	1.36							
17	BLK-2		0.03		2.0	0.1365	0.48							
18	SPK-1		0.09		2.0	0.1410	1.26			0.004	63.00	0.15	0.18	118%
19	SPK-2		0.09		2.0	0.1677	1.02			0.004	63.00	0.15	0.17	113%
20	50-1		0.11		2.0	0.1176	1.95	73.9156	144.06					
21	50-2		0.11		2.0	0.1206	1.85	73.9156	136.68					
22	50-3		0.11		2.0	0.1303	1.73	73.9156	128.20					
23	93-1		0.07		2.0	0.1410	1.00	68.8400	68.55					
24	93-2		0.06		2.0	0.1253	0.99	68.8400	68.46					
25	93-3		0.06		2.0	0.1229	1.01	68.8400	69.57					
26	Drift	1.50	1.47	98%										
27	Wash		ND											
28	92-1		0.02		2.0	0.1409	0.27	80.1586	21.73					
29	92-2		ND		2.0	0.1287	ND	80.1586	ND					
30	92-3		ND		2.0	0.1481	ND	80.1586	ND					
31	51-1		0.16		2.0	0.1078	3.02	66.7527	201.87					

Saw. 117165
AMDT 122094.2
HWI 6329-138
Lower Sample
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
32	51-2		0.17		2.0	0.1332	2.58	66.7527	172.39					
33	51-3		0.17		2.0	0.1328	2.62	66.7527	174.92					
34	52-1		0.42		2.0	0.1429	5.91	94.8862	560.42					
35	52-2		0.76		2.0	0.1419	10.67	94.8862	1012.39					
36	52-3		0.73		2.0	0.1370	10.58	94.8862	1004.27					
37	BLK-1		0.04		2.0	0.1334	0.55							
38	Drift	1.50	1.51	100%										
39	Wash		ND											
40	BLK-2		0.02		2.0	0.1092	0.44							
41	SPK-63-1		0.09		2.0	0.1484	1.21			0.004	63.00	0.15	0.18	119%
42	SPK-63-2		0.09		2.0	0.1370	1.32			0.004	63.00	0.15	0.18	119%
43	SPK 126-1		0.15		2.0	0.1212	2.40			0.004	126.00	0.30	0.29	96%
44	SPK 126-2		0.14		2.0	0.1439	1.91			0.004	126.00	0.30	0.28	91%
45	SPK 126-3		0.14		2.0	0.1085	2.53			0.004	126.00	0.30	0.27	91%
46	Drift	1.50	1.50	100%										
47	Wash		ND											

DDW 7/17/95
AMDT 122094.2
HWI 6329-138
Liver Samples

1995-04-20 09:36 OutPut of : 950420A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-04-20 07:12

Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950420A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00065
a0 = -1.24984

Fluoride L
Calibration order = 2

Correlation : r = 0.99991

Result = a2 * x^2 + a1 * x + a0

a2 = 0.00000
a1 = 0.00020
a0 = 0.00010

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

5

1995-04-20 09:36 OutPut of : 950420A1

Fluoride 1.5

Path number	: 3
Signal type	: Debubbled
Decolor	: Yes
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off
s1 sTandard	: Ignore
s2 sTandard	: Ignore
s3 sTandard	: Ignore
s4 sTandard	: Ignore
s5 sTandard	: Ignore
s6 sTandard	: 0.150
s7 sTandard	: 0.300
s8 sTandard	: 0.600
s9 sTandard	: 1.200
s10 sTandard	: 1.500
Order	: Inverse Logarithm
Dimension	: PPM
start Value	: 500 DU
trigger Limit	: 1800 Sec
Peak shape	: Pointed
stArt ignore	: 60 Sec
eNd ignore	: 120 Sec
Measure window	: 75 %
Filter	: No
Regeneration	: No
formUla	:
output	: ##.###

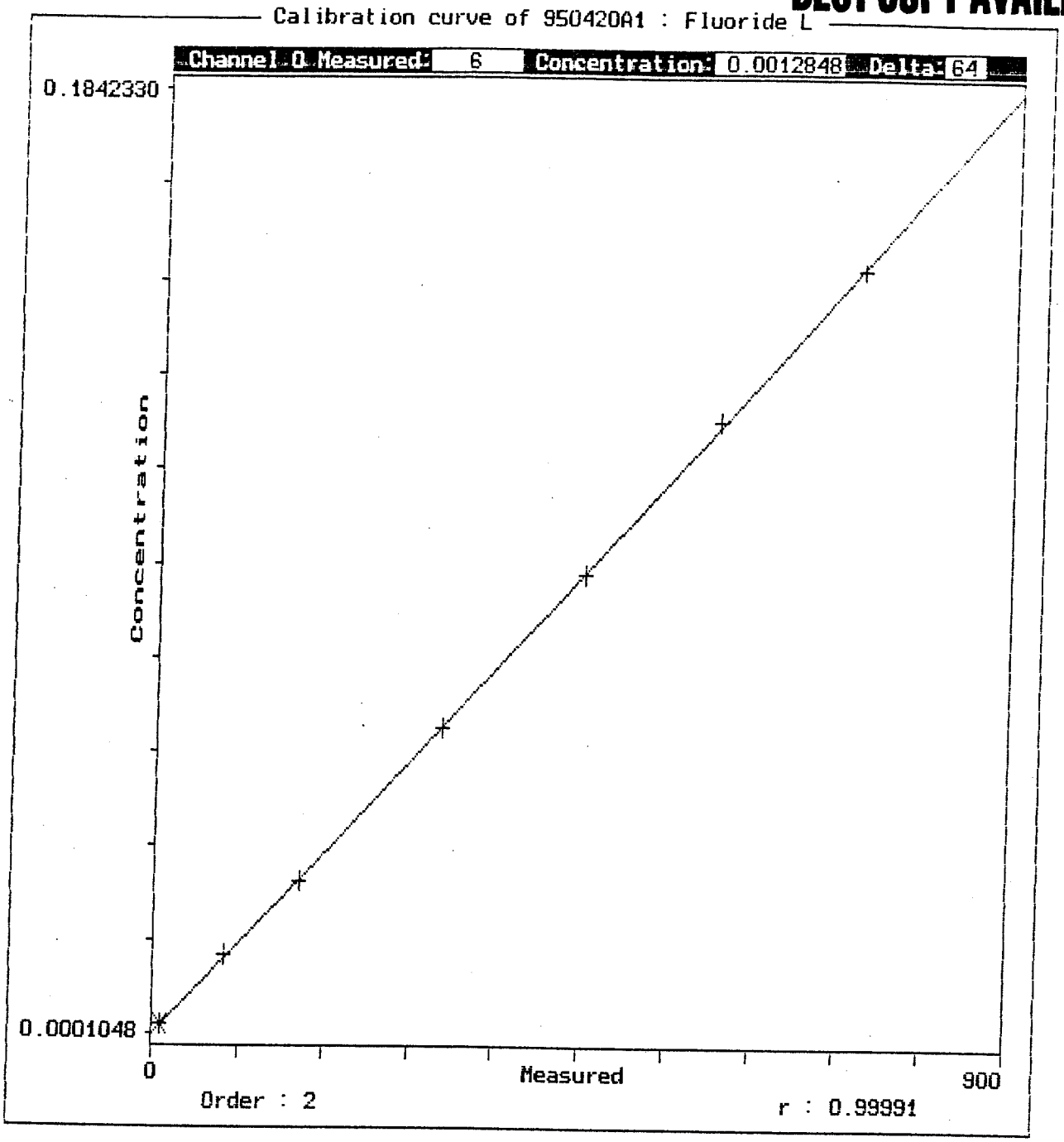
Fluoride L

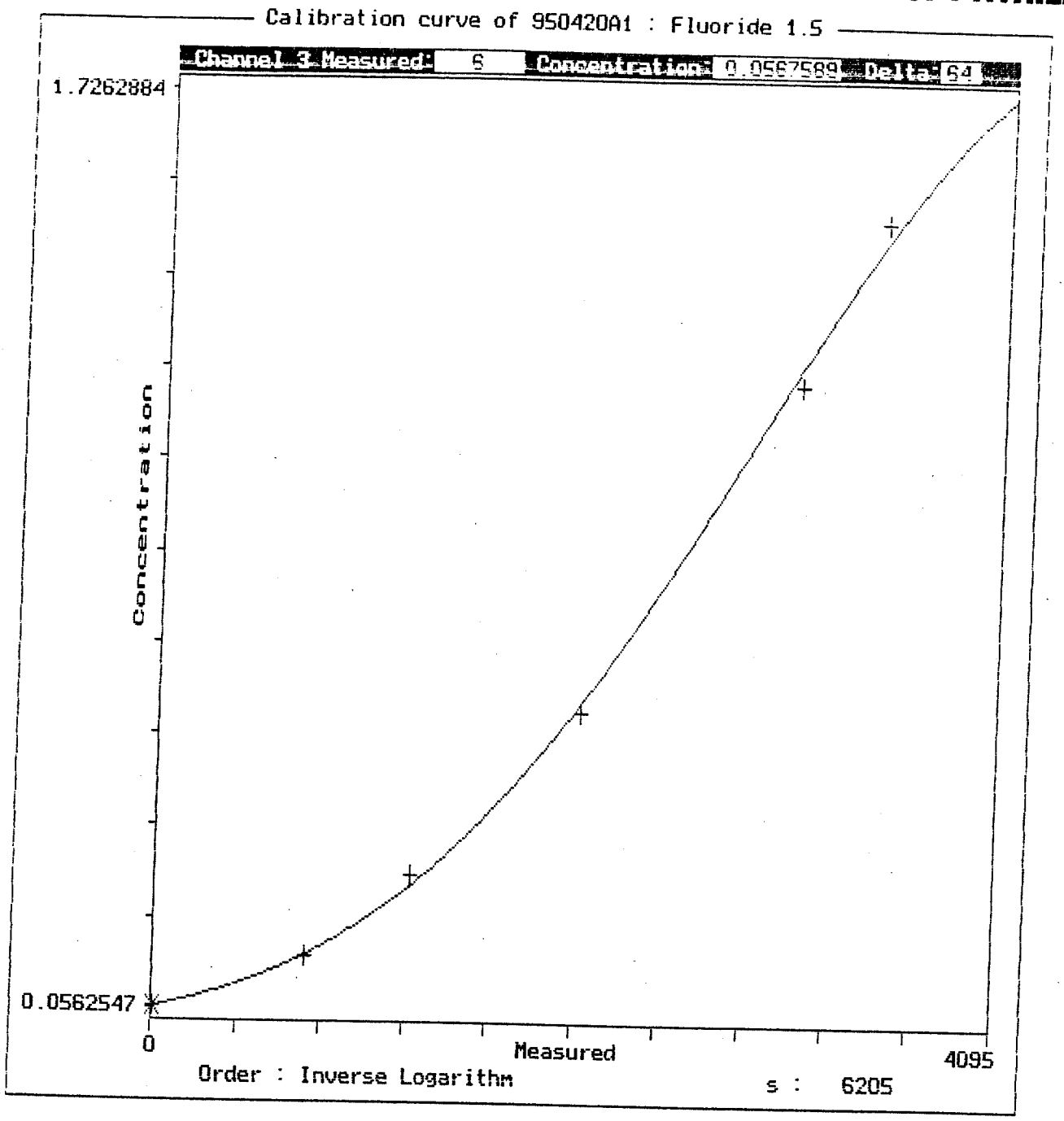
Path number	: 0
Signal type	: Debubbled
Decolor	: No
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off

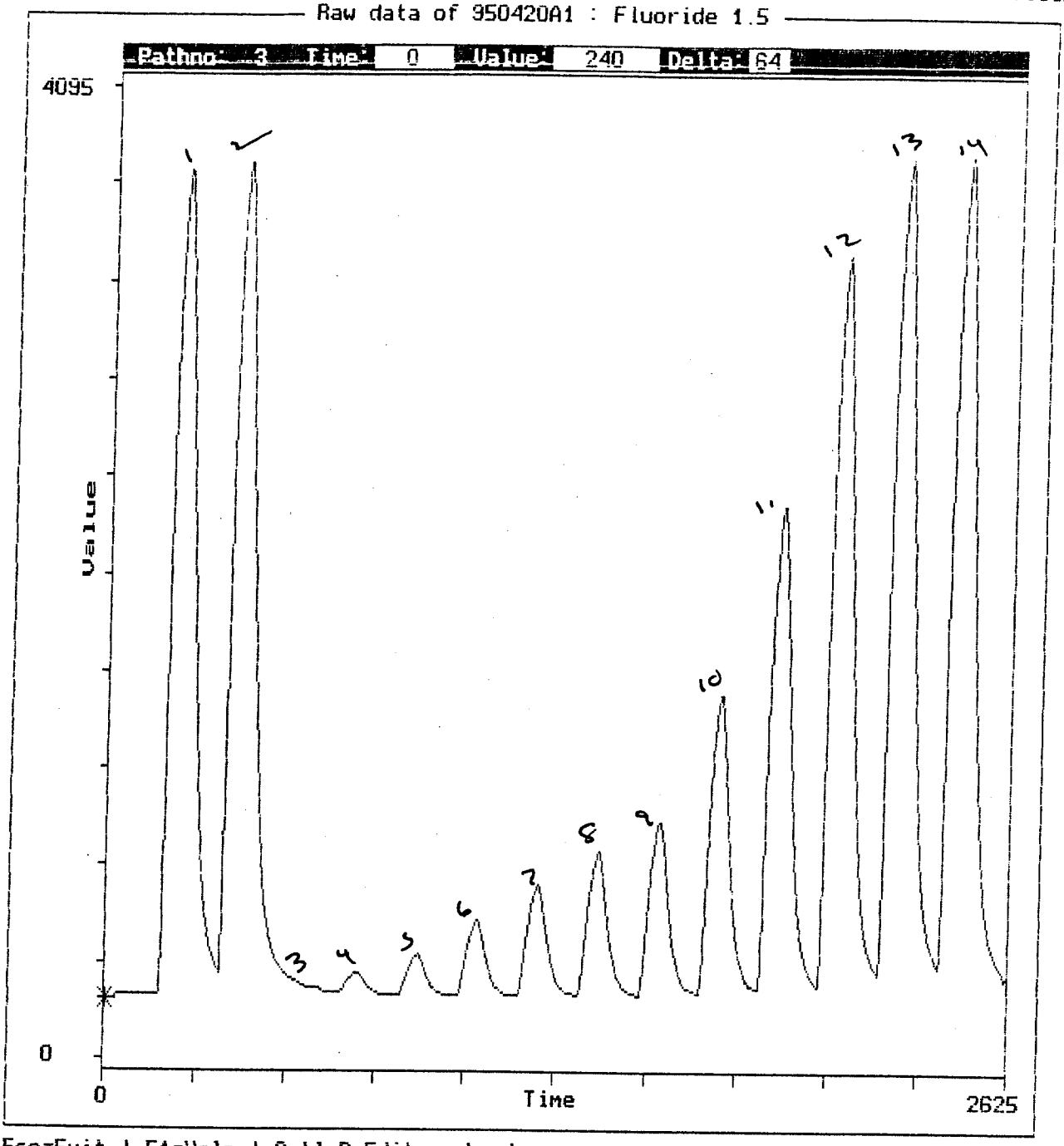
1995-04-20 09:36 OutPut of : 950420A1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

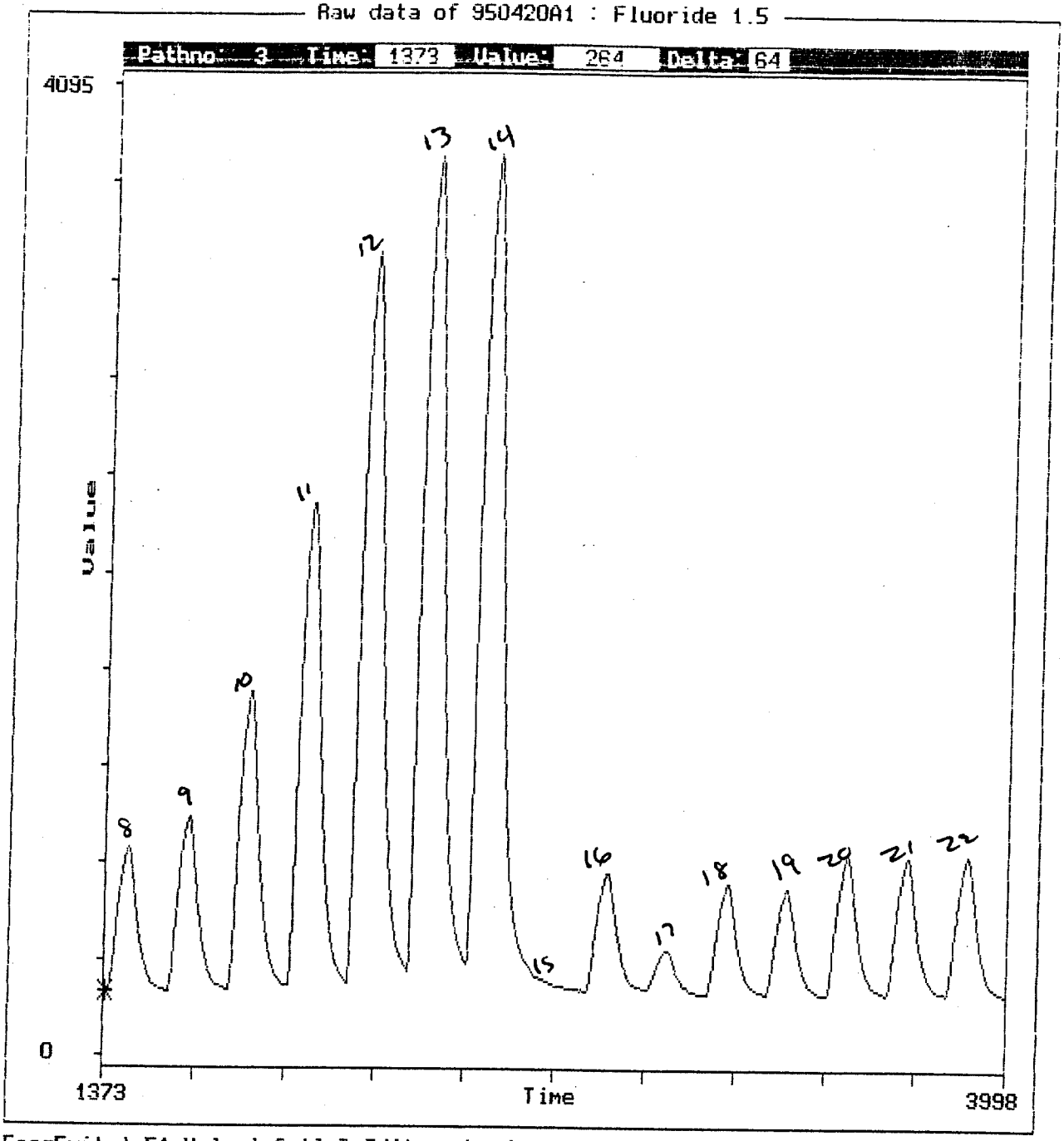
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.056	65	4	0.0001	0
1	t	Tracer	3	1.457	210	4	0.7942	0
2	d	Drift	3	1.470	384	4	0.8006	0
3	w	Wash	3	0.056	626	4	0.0001	0
4	s1	Standard 1	3	0.063	733	4	0.0147	0
5	s2	Standard 2	3	0.070	907	4	0.0302	0
6	s3	Standard 3	3	0.087	1085	4	0.0603	0
7	s4	Standard 4	3	0.106	1261	4	0.0904	0
8	s5	Standard 5	3	0.128	1436	4	0.1190	0
9	s6	Standard 6	3	0.155	1611	4	0.1505	0
10	s7	Standard 7	3	0.282	1785	4	0.2602	0
11	s8	Standard 8	3	0.614	1959	4	0.4429	0
12	s9	Standard 9	3	1.232	2135	4	0.6965	0
13	s10	Standard 10	3	1.467	2311	4	0.7988	0
14	d	Drift	3	1.483	2485	4	0.8066	0
15	w	Wash	3	0.056	2727	4	0.0001	0
16	u	BLK-1	3	0.112	2836	4	0.0986	0
17	u	BLK-2	3	0.071	3011	4	0.0326	0
18	u	SPK-1	3	0.105	3187	4	0.0889	0
19	u	SPK-2	3	0.103	3363	4	0.0857	0
20	u	50-1	3	0.124	3538	4	0.1146	0
21	u	50-2	3	0.122	3714	4	0.1115	0
22	u	50-3	3	0.123	3886	4	0.1130	0
23	u	93-1	3	0.093	4060	4	0.0702	0
24	u	93-2	3	0.088	4238	4	0.0623	0
25	u	93-3	3	0.088	4414	4	0.0621	0
26	d	Drift	3	1.467	4588	4	0.7988	0
27	w	Wash	3	0.056	4740	4	0.0001	0
28	u	92-1	3	0.065	4936	4	0.0191	0
29	u	92-2	3	0.063	5111	4	0.0143	0
30	u	92-3	3	0.061	5285	4	0.0117	0
31	u	51-1	3	0.163	5463	4	0.1586	0
32	u	51-2	3	0.172	5637	4	0.1686	0
33	u	51-3	3	0.174	5813	4	0.1705	0
34	u	52-1	3	0.422	5989	4	0.3468	0
35	u	52-2	3	0.757	6163	4	0.5053	0
36	u	52-3	3	0.725	6339	4	0.4918	0
37	u	BLK-1	3	0.074	6515	4	0.0368	0
38	d	Drift	3	1.507	6689	4	0.8183	0
39	w	Wash	3	0.056	6930	4	0.0001	0
40	u	BLK-2	3	0.067	7034	4	0.0238	0
41	u	SPK-63-1	3	0.106	7214	4	0.0898	0
42	u	SPK-63-2	3	0.106	7390	4	0.0902	0
43	u	SPK 126-1	3	0.151	7566	4	0.1456	0
44	u	SPK 126-2	3	0.143	7740	4	0.1375	0
45	u	SPK 126-3	3	0.143	7914	4	0.1371	0
46	d	Drift	3	1.495	8090	4	0.8123	0
47	w	Wash	3	0.056	8324	4	0.0001	0
wt	rw	RunOut Wash	3	0.056	8565	4	0.0001	0



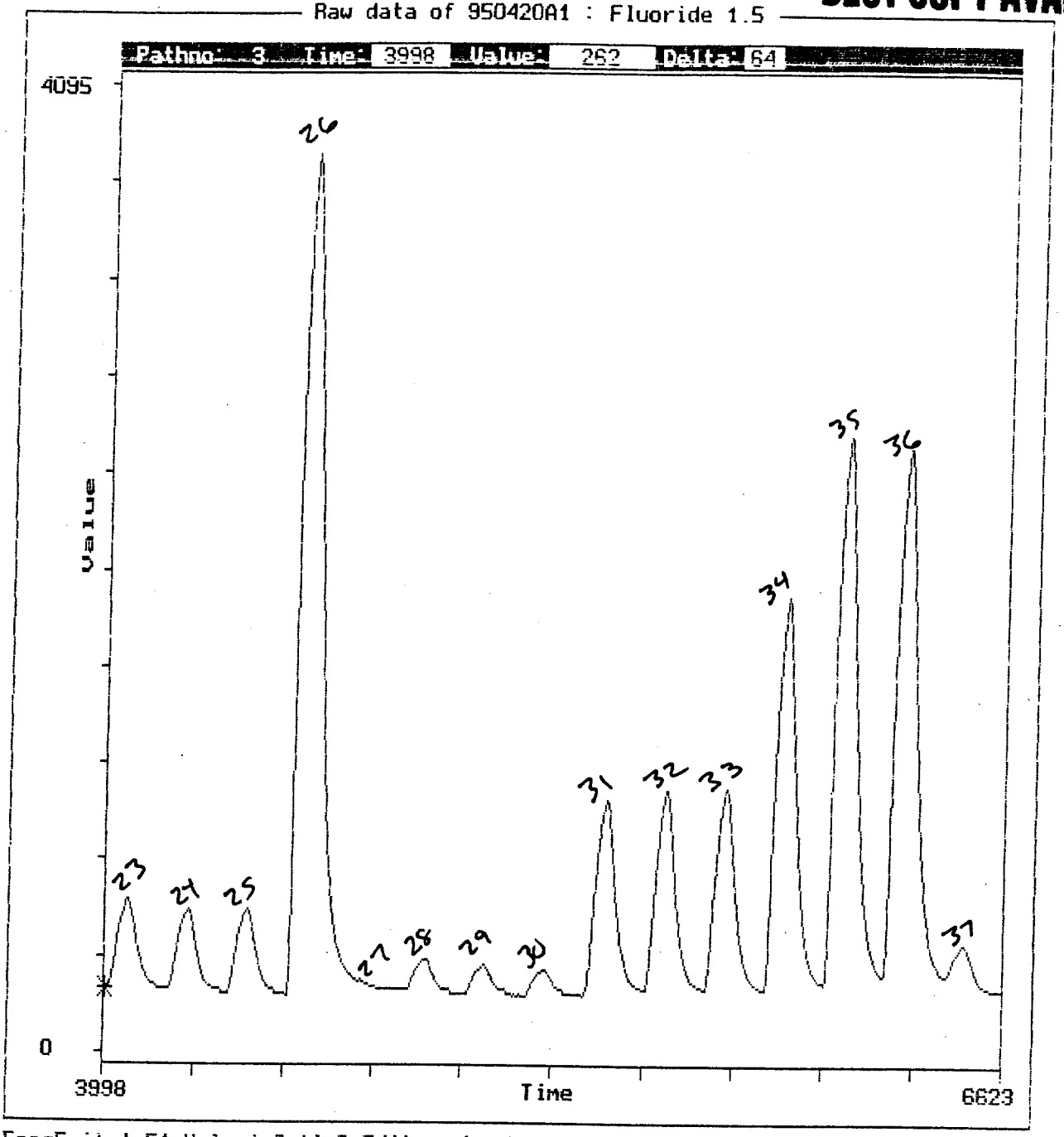


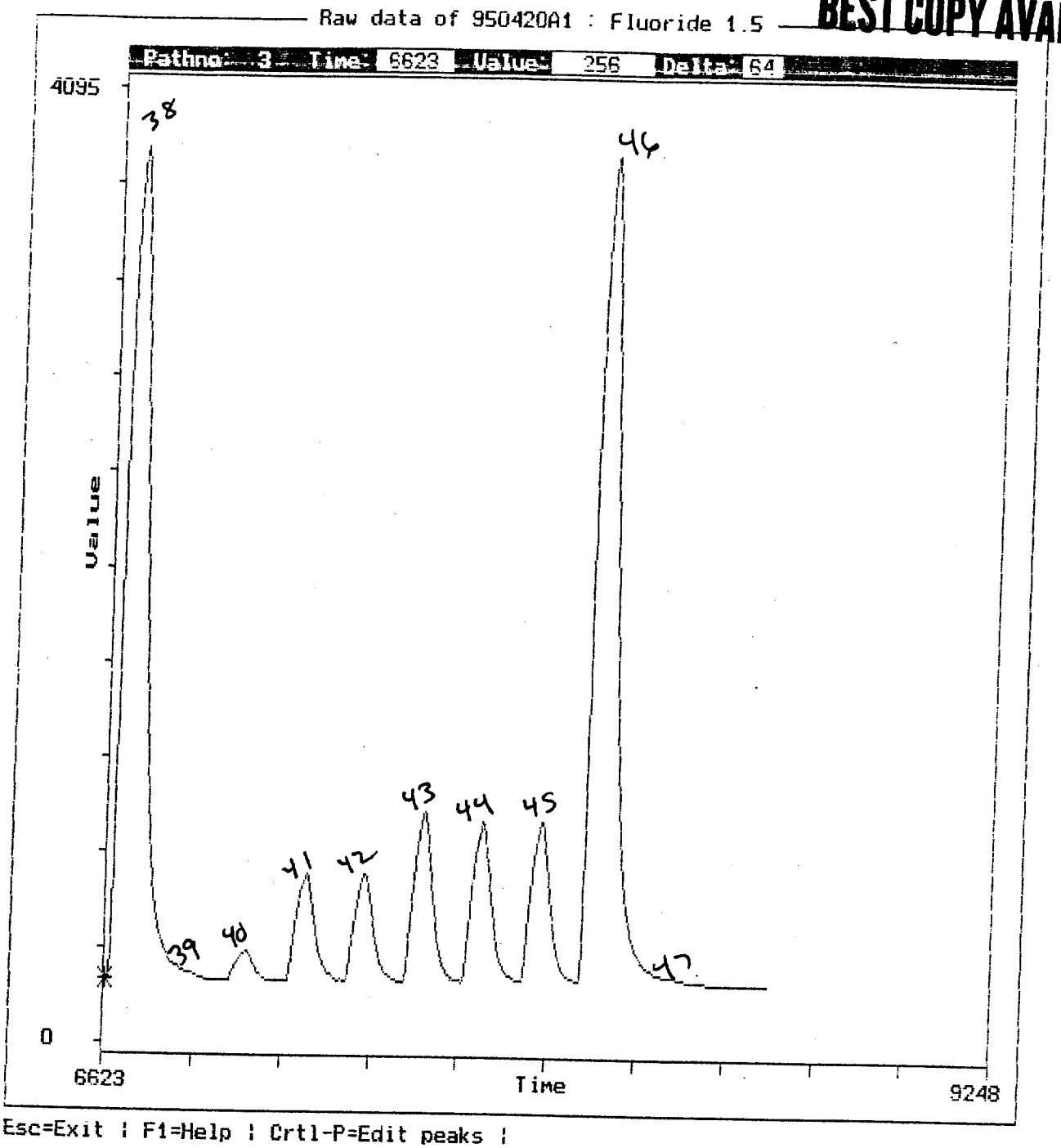


Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



12





Corporate Toxicology Study Outline
July, 1998

Title: FC-129 Preliminary ADME Screen in Rats

Timeline: In Life Start Date: 7/13/98
In Life End Date: 7/17/98

Purpose: This study is designed to provide preliminary information on the absorption, distribution, metabolism and excretion (ADME) of FC-129.

Significance: The results of this ADME screen will be compared to the test results from similar studies on other compounds.

Objective(s): The objective is to rapidly screen FC-129 for its ADME characteristics, in particular the formation of persistent metabolites.

Protocol:
- Charles River Rats /Sprague Dawley/CD/ 415-475 g
- 6 males
- Single dose, oral gavage
- 0 and 5 mg/kg
- 3 rats per group
- Individually housed in matabolism cages
- Commercially available certified Laboratory Rodent Diet #5002 (PMI Feeds, St. Louis, MO) *ad libitum*
- Water *ad libitum*
- Urine and feces collected days 1, 2, 3 and 4 post dose
- Necropsy day 4 post dose
- Liver and sera collected
- Analysis for parent compound and suspected metabolites by Kris Hansen, Ph.D., 3M Environmental Technology and Safety Services.

Sponsor: 3M Specialty Chemicals Division.

Principle Investigator / Location / Cost: Andrew Seacat / 3M Strategic Toxicology Laboratory

Report: Mean concentrations of FC-129 and its metabolites in the collected tissues for each group will be analyzed for differences between means and considered significant at the p=0.05 level.

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

4. PFDS	Perfluorodecanesulfonate

Acute Toxicity

1) Final Report, Acute Dermal Toxicity Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 21, 1988

2) Final Report, Acute Oral Toxicity Study in Rats, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 25, 1988

3) Final Report, Primary Eye Irritation/Corrosion Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 20, 1988

4) Final Report, Primary Dermal Irritation/Corrosion Study in Rabbits, Hazelton Laboratories America, Inc., 3M Reference No. T-4102, Sample No. T837389-410 754, January 21, 1988

Genotoxicity

1) Mutagenicity Test with T-6357 in the *Salmonella – Escherichia coli* / Mammalian-Microsome Reverse Mutation Assay, Corning Hazleton, Inc. (CHV), Project No. 17387-0-409, 3M Reference No. T-6357, FC-120, April 1, 1996

2) Mutagenicity Test on T-6357 in an In Vivo Mouse Micronucleus Assay, Corning Hazleton, Inc. (CHV), Project No. 17387-0-409, 3M Reference No. T-6357, FC-120, April 23, 1996

Pharmacokinetic Studies

1) Final Report, Analytical Report and Single-Dose Dermal Absorption / Toxicity Study of T-6052 in Rabbits, Hazleton Wisconsin, Inc., Project No. HWI 6329-135, 3M Reference No. FC-120, T-6052 (0.02 % in water), November 20, 1995

2) Single-Dose Dermal Intravenous Pharmacokinetic Study of T-6052 in Rabbits, Hazleton Wisconsin, Inc., Project No. HWI 6329-134, 3M Reference No. T-6052 (0.02 % in water), November 20, 19995

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

Pre-1976 Studies (bibliography only)

1) Acute Oral Toxicity Study with T-1019 in Male Albino Rats, Industrial Bio-Test Laboratories, Inc., Project No. 601-05394, 3M Reference No. T-1019, August 6, 1974

2) Skin Irritation, Eye Irritation, Acute Oral LD50, WARF Institute, Inc., Project No. 4053863, 3M Reference No. T-992, May 24, 1974

3) Acute Oral Cholinesterase Study with T-1019 in Male Albino Rats, Industrial Bio-Test Laboratories, Inc., Project No. 601-05394, 3M Reference No. T-1019, August 6, 1974

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

ROGER G. PERKINS
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 70905762
SAMPLE ENTERED: 09/25/87
REPORT PRINTED: 01/21/88

SAMPLE: T-4102

PURCHASE ORDER NUMBER: T837389-410 754

ENCLOSED: ACUTE DERMAL TOXICITY STUDY IN RABBITS (OECD GUIDELINES)

- Key Personnel
- Method
- Summary
- Individual Pathology Comments
- References
- Pathology Report
- Raw Data Appendix

SIGNED: Steven M. Glaza ..... DATE 1-21-88

STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

SAMPLE NUMBER: 70905762

SAMPLE: T-4102

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director

Calvin L. Horton
Group Leader
Support Services

Sharen L. Howery
Report Coordinator

Quality Assurance

Debra Curley Arndt
Manager

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Robert Salava
Senior Section Supervisor

Dennis Hoffman
Group Leader
Necropsy

Anne Mosher
Group Leader
Pathology Data

Laboratory Animal Veterinarian

Cindy J. Cary, DVM
Diplomate, ACLAM

SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN

Objective: To assess the systemic toxicity and relative skin irritancy of a test material when it is applied to the skin according to the Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals.

Test Material: T-4102

Physical Description:	Dark amber liquid
Purity and Stability:	Sponsor assumes responsibility for purity and stability determinations.
Storage and Retention:	The test material was stored at room temperature. Any unused material will be discarded according to HLA Standard Operating Procedure.
Safety Precautions:	Normal handling procedures were used according to HLA Standard Operating Procedure.

Test Animal: Young adult rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days. Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals". If variations from the prescribed environmental conditions existed, they were documented and considered to have no effect on the study outcome. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

Five male and five female acclimated rabbits, weighing between 2097 g and 2345 g, were chosen at random, treated, and maintained during the observation period as specified for the acclimation period. Test animals were identified by animal number and corresponding ear tag. Approximately twenty-four hours before test material application, each rabbit's back was shaved with an electric clipper. The shaved area made up approximately 20% of the total body surface.

Reason for Species Selection: Historically, the New Zealand White albino rabbit has been the animal of choice due to the large amount of background information on this species.

Preparation of Test Material: The sample was dosed as received. An individual dose was calculated and weighed out based upon each animal's body weight at initiation.

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SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN (CONTINUED)

Treatment: The test material was applied to each animal's back at a dose level of 2.0 g/kg. The area of application was covered with a 10 x 10-cm gauze patch secured with paper tape and overwrapped with Saran Wrap and Elastoplast tape. Twenty-four hours later the bandages were removed and the backs were washed with lukewarm tap water and wiped with disposable paper towels. Collars were applied to restrain the test animals during the 24-hour exposure period.

Reason for Route of Administration: Historically, this is the route of choice is based on the method of Draize.

Observations: Each animal was observed for clinical signs and mortality at 1, 2.5 and 4 hours after test material administration. Thirty minutes after removal of the test material the initial dermal irritation reading was made. Subsequent readings of dermal irritation were made on Study Days 3, 7, 10 and 14. The animals were observed daily for clinical signs and twice daily (morning and afternoon) for mortality. The animals were weighed just prior to test material application. Body weights were taken again at 7 days and at study termination or at death.

Pathology: At study termination, surviving animals were euthanatized. All animals, whether dying on test or euthanatized at termination, were subjected to a gross necropsy examination and all abnormalities were recorded. After necropsy, animals were discarded and no tissues were saved.

Statistical Methods: Other than average body weights, no other statistical method was performed.

Location of Raw Data and Final Report: The raw data and a copy of the final report will be retained in the archives of HLA.

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SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 11/03/87

Method of Administration: Dermal Application

Date Test Started: 11/16/87 Date Test Completed: 11/30/87

Estimated Dermal LD50: Male - Greater than 2.0 g/kg of body weight
Female - Greater than 2.0 g/kg of body weight

MORTALITY SUMMARY (NUMBER OF DEATHS)

Dose Level (G/KG)	Hours 0 - 4		Days 1		2		3		4		5		6		7-14		Total		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both
2.0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1/5	0/5	1/10

Average Body Weights (g)

	Initial	Day 7	Terminal
Male	2187	1784	1671
Female	2193	2148	2374

SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN (CONTINUED)

Clinical Signs
(No. of Animals Affected)

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Appeared Normal	5	5	5	5	4	4	4	0	0	0	0	0	0	0	0	0	0
Thin appearance	0	0	0	0	1	1	0	0	0	1	1	1	3	3	3	3	3
Hypoactivity	0	0	0	0	1	1	0	0	1	3	3	3	3	3	3	2	3
Soft stool	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1
Loss of appetite	0	0	0	0	0	0	0	2	3	3	3	3	3	3	3	2	2
Few feces	0	0	0	0	0	0	0	3	3	3	3	3	3	3	3	3	3
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1	0
Death	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1*
Females																	
Appeared Normal	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	5	5
Few feces	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
Loss of appetite	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0

* Upon transferring animals to the necropsy area, Animal No. F20575 appeared to go into a subconvulsive state for approximately 3 to 5 minutes. At this time the animal was necropsied as a moribund sacrifice.

Comments: Dermal irritation observed during the study consisted of slight to moderate erythema and desquamation and slight edema and fissuring.

SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN (CONTINUED)

PATHOLOGY

Dose Level: 2.0 g/kg of body weight

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
F20455	M	-	14	Perianal stains - brown; animal appears thin.
F20491	M	-	14	Animal thin.
F20575	M	-	14	Perinal stains - tan; animal appears thin.
F20493	M	4	-	Treated skin - diffusely red; perineum - stained brown; colon - contents unformed; cecum - contents dry to impacted; cecum and ileum - serosa has multiple red areas, up to 5.0 x 2.0 cm.
F20564	M	-	14	Colon - contains clear, frothy fluid; perianal stains - green; animal appears thin.
F20535	F	-	14	Perianal stains - green; subcutaneous tissue underneath treated skin - multiple red, pinpoint foci.
F20558	F	-	14	Perianal stains - dark brown; left axillary lymph node - diffusely dark red; animal appears thin.
F20570	F	-	14	Subcutaneous tissue underneath treated skin - multiple, red areas, up to 1.2 x 0.8 cm; treated skin - thickened.
F20465	F	-	14	Subcutaneous skin underneath treated skin - multiple, red, pinpoint foci.
F20485	F	-	14	Subcutaneous skin underneath treated skin - multiple, red areas, up to 0.2 x 0.1 cm.

SAMPLE NUMBER: 70905762

SAMPLE: T-4102

OECD DERMAL SCREEN (CONTINUED)

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 402, Acute Dermal Toxicity, Adopted May 12, 1981.

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

3. OECD Principles of Good Laboratory Practice, Annex 2, C(81)30 (Final).

4. NIH Publication No. 85-23 (revised 1985).

HLA LAB NO. 70905762
PATHOLOGY REPORT
Acute Dermal Toxicity

Ten rabbits (five males, five females) were necropsied. One male died on test and the remaining animals were euthanatized at the termination of the study. The dose level, day of death, and gross observations recorded for each animal are on page 7 of this report. The subcutaneous tissue of the treated skin of four females had multiple red foci or areas of variable size. The treated skin of one of these appeared to be thickened. Several animals were noted to be thin. All other observations were considered incidental and unrelated to treatment. The treated skin of the died on test male was diffusely red. This animal's death was attributed to enteritis of unknown etiology and unrelated to treatment.

Thomas E. Palmer, PhD
Pathologist

1-21-88
Date

(1734mcs)

HLA No. 7090562

Personnel Signature Sheet
Acute Toxicology

Name	Job Title	Signature	Initials
Becky Beckwith	Sr. Lab. Animal Asst.	Becky Beckwith	BB
Steve Beloungy	Lab. Animal Asst.	Steve Beloungy	SB
Ken Bridges	Sr. Lab. Animal Asst.	Ken Bridges	KB
Pat Crary	Sr. Lab. Animal Asst.	Pat Crary	PC
Shane Eith	Lab Animal Asst.	Shane Eith	SE
Shawn Frazier	Lab Animal Asst.	Shawn Frazier	SF
Steven M. Glaza	Group Leader	Steven M. Glaza	SG
Molly Hahn	Admin. Clerk	Molly Hahn	MH
Kevin Hamilton	Lab. Animal Asst.	Kevin Hamilton	KH
Jeff Hicks	Sr. Lab. Animal Asst.	Jeff Hicks	JH
Calvin Horton	Group Leader	Calvin Horton	CH
Sharen L. Howery	Administrative Asst.	Sharen L. Howery	SLH
Gregory Johnson	Lab. Animal Tech.	Gregory Johnson	GJ
Paul Krebs	Lab. Animal Asst.	Paul Krebs	P.K.
Wayne Madison	Section Supervisor	Wayne A. Madison	WAM
Scott McConnell	Lab. Animal Tech.	Scott McConnell	SMc
Shelley McConnell	Lab. Animal Tech.	Shelley McConnell	SMc
Dawn Conant	Sr. Lab. Animal Asst.	Dawn Conant	DC
Don Mavis	Lab. Animal Asst.	Don Mavis	DM
Robin Olson	Lab. Animal Asst.	Robin Olson	RO
Patricia Padgham	Team Leader	Patricia Padgham	PP
Joseph J. Daun	Lab. Animal Asst.	Joseph J. Daun	JJD
Michael Patzka	Lab. Animal Asst.	Michael Patzka	MP
John Paulson	Sr. Lab. Animal Asst.	John Paulson	JW
Jane Polnow	Lab. Animal Tech.	Jane Polnow	JP
Dennis B. Steiner	Lab. Animal Tech.	Dennis B. Steiner	DS
Michael Thesing	Lab. Animal Asst.	Michael Thesing	MT
Paula G. Vangen	Administrative Asst.	Paula G. Vangen	PGV
Albert Olson	Manpower	Albert Olson	AO
Jim Jirschele	LTE	Jim Jirschele	JJ
Ben Haley	Sr. Lab Animal Asst.	Ben Haley	BH
Eileen M. McConnell	Admin. Clerk	Eileen M. McConnell	EMM
Annette R. Turner	Manpower	Annette R. Turner	ART

HLA: 70905762

ACUTE DERMAL APPLICATION LD_{50} IN NEW ZEALAND WHITE RABBITS

Test Material: T-4102
Physical Description: dark Amber liquid Vehicle: NA
Dosage Level (g/kg): 2.0g/Kg Date Animals Clipped: 11/5/87 Technician: BB
Date Animal Received: 11/3/87 Source: Hazleton Research Products Room No.: 259 Moved to 161E on 11/19/87. pgr 11/19

Moved to 161C on 11-21-87. SLW 11-21-87

DOSE CALCULATIONS				COMPOUND PREPARATION WEIGHTS		
Animal Number	Body Weight (kg)	Dose Level (g/kg)	Dose Animal (g)	Tare Weight (g)	Total Weight (g)	Sample Weight (g)
F2-0455	2.196	2.0	4.39	5.43	9.82	4.39
0491	2.114		4.23	5.48	9.71	4.23
~~0554~~ 0575	~~2.323~~ 2.333 ③		4.65	5.50	10.15	4.65
0493	2.160		4.32	5.31	9.63	4.32
0564	2.143		4.29	5.49	9.78	4.29
0535	2.118		4.24	5.46	9.70	4.24
0558	2.184		4.37	5.40	9.77	4.37
0570	2.097		4.19	5.44	9.63	4.19
0465	2.222		4.44	5.49	9.93	4.44
0485	2.345	✓	4.69	5.40	10.09	4.69

Calculated by: CS Date: 11-16-87 Conducted by: CS Date: 11-16-87
Verified by: BB Date: 11-16-87 Approved by: BB Date: 11-16-87
Scale Used: Sartorius 2811002

Animal Body Weights (g)

Animal Number	Skin Prep	Sex	Study Day 0	7	14
F2-0455	I	♂	2196	1894 KB	2090 KB
0491	I	♂	2114	1712 KB	1470 KB
~~0554~~ 0575 ①	I	♂	2323	807 KB	1562 KB
0493	I	♂	2160	Found DEAD 11/20/87 B.W. 1704g. KB 11/	
0564	I	♂	2143	1723 KB	1562 KB
0535	I	♀	2118	1933 KB	2211
0558	I	♀	2184	1896 KB	2185
0570	I	♀	2097	2361	2603
0465	I	♀	2222	2224	2325
✓ 0485	I	♀	2345	2327 KB	2547
Technician	BB	BB	BB	KB	KB
Date 1987	11/16	11/16	11/16	11/23	11/30
Scale Used		Ktron	1348	③ ~~5228~~ 5228	5228

① Entry errors. BB 11/16/87

② ILLEGIBLE ENTRY CORRE 12/8/87. KB 12/8/87.

③ ENTRY ERROR CORRECTED late CS 1-21-88

I - Intact
A - Abraded (with a clipper blade)
NA - Not Applicable

Reviewed By [signature] Date 12-16-87

(00261/vmt)

HLA: 70905762

MORTALITY RECORD

Test material: T-4102 Dose level: 2.0g/Kg

Observation Period (Days)

Animal Number	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
FZ-0455	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0491	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	✓	✓	✓	✓
①0575 ~~0554~~	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0493	✓	✓	✓	✓	✓	✓	X																					→
0564	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0535	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0558	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0570	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0465	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0485	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Technician	BB	BB	KB	KB	CW	CW	KB	KB	SMC	NP	KB	NP	KB	KB	BB	BB	BB	AH	QW	CW	[illegible]	[illegible]	CW	CW	NP	NP	KB	KB
Date 1987	11/17	11/17	11/18	11/18	11/19	11/19	11/20	11/20	11/21	11/21	11/22	11/22	11/23	11/23	11/24	11/24	11/25	11/25	11/26	11/26	11/27	11/27	11/28	11/28	11/29	11/29	11/30	11/30

NA - Not applicable.
X - Dead.
✓ - Alive.

①Entry Error BB 11/16/87

Reviewed by: [signature]

Date: 12-6-87

(00261/vmt)

HLA: 70905762

OBSERVATIONS (Individual)

Dose level: 2.0g/Kg Test material: T-4102

Male/Female

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
F2-0455	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–	–	–	–	–	–	–
	Soft stool	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	–	–	✓	✓
	Loss of appetite	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	–	–
F2-0491	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–	–	–	–	–	–	–
	Few feces	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	HYPOACTIVE	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	APPEARS THIN	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	diarrhea	–	–	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	–
F2-~~0554~~① 0575	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–	–	–	–	–	–	–
	Few feces	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	LOSS OF APPETITE	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓
	HYPOACTIVE	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	–	✓
	diarrhea	–	–	–	–	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–
	Appears Thin	–	–	–	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	2✓* ③
F2-0493	Appeared Normal	✓	✓	✓	✓	✓	–	–	–										
	APPEARS THIN	–	–	–	–	–	✓	✓	–										
	HYPOACTIVE	–	–	–	–	–	✓	✓	–										
	DEAD	–	–	–	–	–	–	–	✓										
F2-0564	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–	–	–	–	–	–	–
	Few feces	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Loss of appetite	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	–	✓	✓	✓
	HYPOACTIVE	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Appears Thin	–	–	–	–	–	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓
	Deaths					0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Collected by	BB	BB	BB	BB	BB	KB	CF	KB	SMK	KB	KB	BB	BB	SW	CM	CF	RJ	KB
	Date 1987	11/16	11/16	11/16	11/16	11/17	11/18	11/19	11/20	11/21	11/22	11/23	11/24	11/25	11/26	11/27	11/28	11/29	11/30

2. UPON TRANSFERRING ANIMALS TO ③ NECROPSY AREA ANIMAL NUMBER F20575 APPEARED TO GO INTO A SUB-CONVULSIVE STATE. HE REMAINED IN THIS MANNER FOR APPROXIMENTLY 3-5 MINUTES, AT THIS TIME HE WAS TAKEN TO NECROPSY AS A MORIBUND SACRIFICE. KB 11/30/87

- Surviving animals submitted for terminal necropsy. Technician KB Date 11/30/87

- Surviving animals designated for sacrifice and discard. Technician NA Date NA

✓ Indicates condition exists
Sl Slight.
– Condition not evident.
* Found dead, P.M. check.

Reviewed by: Date: 12/6/87

①Entry Error BB 11/16/87

③Symbol Used was used as a found dead for P.M. check

HLA: 70905762

OBSERVATIONS (Individual)

Dose level: 2.0g/Kg Test material: T-4102

Male/~~Female~~

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
F2-0535	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	—	✓	✓	✓
F2-0558	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	—	-	-	-	-	-	—	-	✓	✓
	Few feces	—	—	—	—	—	—	—	—	✓	✓	✓	✓	✓	✓	✓	✓	—	-
	Loss of appetite	—	—	—	—	—	—	—	—	✓	✓	✓	✓	✓	✓	✓	✓	—	-
F2-0570	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F2-0465	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F2-0485	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Deaths																		
	Collected by	BB	BB	BB	BB	BB	KB	CW	KB	CW	KB	KB	BB	BB	CW	CW	CW	CW	KB
	Date 1987	11/16	11/16	11/16	11/16	11/17	11/18	11/19	11/20	11/21	11/22	11/23	11/24	11/25	11/26	11/27	11/28	11/29	11/30

NA - Not Applicable, KB 12/8/87.

- Surviving animals submitted for terminal necropsy.
  Technician KB
  Date 11/30/87

- Surviving animals designated for sacrifice and discard.
  Technician NA
  Date NA

✓ Indicates condition exists
SI Slight.
- Condition not evident.
* Found dead, P.M. check.

Reviewed by:
Date: 12/8/87

HLA: 70905762

ACUTE DERMAL IRRITATION OBSERVATIONS

Test material: T-4102

Dose level: 2.0g/Kg

Observation Period (Days)

1987	Males 1 (11/17)	3 (11/19)	7 (11/23)	10 (11/26)	14 (11/30)	Females 1 (11/17)	3 (11/19)	7 (11/23)	10 (11/26)	14 (11/30)
Animal No.: F2-0456 Intact/Abraded										
Erythema	1	1	1	1	1	1	1	1	1	1
Edema	0	1	1	1	1	1	1	1	1	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	1	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0
Animal No.: F2-0491 Intact/Abraded						**Animal No.: F2-0558 Intact/Abraded**				
Erythema	1	1	1 [1]	1	1	1	1	1	1	0
Edema	0	1	0	0	0	0	1	0	0	0
Atonia	0	0	0	0	1	0	0	0	③ X 0	0
Desquamation	0	0	0 [2][3]	2	2	0	0	0	③ B 1	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	1	1	0	0	0	1	0
Animal No.: F2-0554 (0575 Entry Error BB 11/16/87) Intact/Abraded						**Animal No.: F2-0570 Intact/Abraded**				
Erythema	0	0	0	0	0	1	1	1	0	0
Edema	0	0	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	1	1	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0
Animal No.: F2-0493 Intact/Abraded						**Animal No.: F2-0465 Intact/Abraded**				
Erythema	0	0	Found DEAD			2	2	2	1 [2][3]	1
Edema	0	0	11/20/87 KB			1	1	1	0	0
Atonia	0	0				0	0	0	0	0
Desquamation	0	0				0	0	0	1	0
Coriaceousness	0	0				0	0	0	0	0
Fissuring	0	0				0	0	0	1	0
Animal No.: F2-0564 Intact/Abraded						**Animal No.: F2-0485 Intact/Abraded**				
Erythema	1	1	1 [1][2]	1	1	1	1	1	③ X 0	0
Edema	0	0	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0 [1][2]	1	1	0	0	0	③ X 0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	1	1	0	0	0	③ X 0	0
Technician	BB	CS	KB	G.W.	KB	BB	CS	KB	G.W.	KB
Date 1987	11/17	11/19	11/23	11/26	11/30	11/17	11/19	11/23	11/26	11/30

A - Subcutaneous hemorrhage.
B - Blanching.
C - Scab formation.
D - Eschar.
E - Exfoliation.
~~F - Desquamation.~~ KB 11/23/87 ①

① ENTRY ERRORS KB 11/23/87
② WRONG SCORES ENTERED. KB 11/23/87
③ Entry error, G.W., 11-28-87

Reviewed by: [signature] Date: 12-6-87

(00261/vmt)

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Chemical & BioMedical Sciences Division
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FINAL REPORT

ROGER G. PERKINS
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 70905761
SAMPLE ENTERED: 09/25/87
REPORT PRINTED: 01/25/88

SAMPLE: T-4102

PURCHASE ORDER NUMBER: T837389-410 754

ENCLOSED: ACUTE ORAL TOXICITY STUDY IN RATS (OECD GUIDELINES)

- o Key Personnel
- o Method
- o Summary
- o Individual Pathology Comments
- o References
- o Pathology Report
- o Raw Data Appendix

SIGNED: Steven M. Glaza
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

1-25-88
DATE

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SAMPLE NUMBER: 70905761

SAMPLE: T-4102

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director

Calvin L. Horton
Group Leader
Support Services

Sharen L. Howery
Report Coordinator

Quality Assurance

Debra Curley Arndt
Manager

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Robert Salava
Senior Section Supervisor

Dennis Hoffman
Group Leader
Necropsy

Anne Mosher
Group Leader
Pathology Data

Laboratory Animal Veterinarian

Cindy J. Cary, DVM
Diplomate, ACLAM

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN

Objective: To determine the acute oral toxicity produced when a test material is administered by the oral route (gavage) to rats according to the Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals.

Test Material: T-4102

Physical Description:	Dark amber liquid
Purity and Stability:	Sponsor assumes responsibility for purity and stability determinations.
Storage and Retention:	The test material was stored at room temperature. Any unused material will be discarded according to HLA Standard Operating Procedure.
Safety Precautions:	Normal handling procedures were used according to HLA Standard Operating Procedure.

Test Animal: Young adult albino rats of the Sprague-Dawley strain were procured, separated by sex, maintained in group cages in temperature- and humidity-controlled quarters, provided continuous access to Purina Rodent Chow and water, and held for an acclimation period of at least 7 days. Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals". If variations from the prescribed environmental conditions existed, they were documented and considered to have no effect on the study outcome. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

Acclimated animals were chosen at random for the study. Test animals were housed by sex in groups of five and identified by animal number and corresponding ear tag. Food and water were available ad libitum throughout the study, except for an overnight period just before test material administration when food, but not water, was withheld.

Reason for Species Selection: The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Method: Five male and five female rats weighing from 200 to 284 g were used for each dose level. The study consisted of two dose levels (0.50 and 5.0 g/kg).

Preparation and Administration of Test Material: An individual dose was calculated for each animal based upon its fasted body weight and administered undiluted by gavage.

The dose volume of the test material varied per dose level based upon an average bulk density of 1.06 g/ml.

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

Reason for Route of Administration: This is the method for administering a known quantity of test substance and has been the route of choice historically.

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours after test material administration. The animals were observed daily thereafter for 14 days for clinical signs and twice a day for mortality.

All animals were weighed just before test material administration, at 7 days and at study termination (or at death).

Pathology: At study termination surviving animals were euthanatized. Animals which died on study or euthanatized at study termination were subjected to a gross necropsy examination and abnormalities recorded. Following necropsy, animals were discarded and no tissues were saved.

Statistical Methods: Other than average body weights, no other statistical method was performed.

Location of Raw Data and Final Report: The raw data and a copy of the final report will be retained in the archives of HLA.

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

SUMMARY

Test Animal: Albino Rats - Sprague-Dawley strain
Source: Charles River Laboratories, Inc., Portage MI
Date Animals Received: 09/08, 10/12, and 10/26/87

Method of Administration: Oral Gavage

Date Test Started: 10/26/87 Date Test Completed: 11/20/87

Estimated Oral LD50: Male - Between 0.50 and 5.0 g/kg of body weight
Female - Between 0.50 and 5.0 g/kg of body weight

	Dose Level (g/kg)	Average Body Weights (g) Initial	Day 7	Terminal	Mortality (Number Dead/Number Dosed)
Male	0.50	272	273	339	2/5
	5.00	255	---	---	5/5
Female	0.50	210	198	238	0/5
	5.00	216	---	---	5/5

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

CLINICAL SIGNS
(No. of Animals Affected)

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DOSE LEVEL: 0.50 g/kg of body weight																	
Males																	
Appeared normal	5	5	5	5	5	5	4	0	0	3	3	3	3	3	3	3	3
Aggressive behavior	0	0	0	0	0	0	3A	1	1	0	0	0	0	0	0	0	0
Hypersensitivity to touch	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0
Hypoactivity	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0
Intermittent clonic convulsions	0	0	0	0	0	0	0	0	1A	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	2B	0	0	0	0	0	0	0	0	0	0
Females																	
Appeared normal	5	5	5	5	5	5	0	0	0	3	3	3	4	4	4	4	4
Aggressive behavior	0	0	0	0	0	0	5	4	0	0	0	0	0	0	0	0	0
Hypoactivity	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Hypersensitivity to touch	0	0	0	0	0	0	0	2	4	1	1	1	0	0	0	0	0
Ataxia	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Intermittent clonic convulsions	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Alopecia - back region	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1

A - Sign noted after a.m. observation.
B - One animal found dead after a.m. observation.

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN

(CONTINUED)

CLINICAL SIGNS (Continued)
(No. of Animals Affected)

DOSE LEVEL: 5.0 g/kg of body weight

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males																	
Hypoactivity	5	5	5	5	2	0	0	-	-	-	-	-	-	-	-	-	-
Miosis	4	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-
Ataxia	2	1	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	3	1	0	0	-	-	-	-	-	-	-	-	-	-
Lacrimation	0	0	0	2	0	0	0	-	-	-	-	-	-	-	-	-	-
Yellow-stained genital region	0	0	0	0	1	0	0	-	-	-	-	-	-	-	-	-	-
Pain reflex absent	0	0	0	0	1	0	0	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-
Tonic convulsions	0	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-
Excessive salivation	0	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	1A	2	1	1	-	-	-	-	-	-	-	-	-	-
Females																	
Appeared normal	0	0	0	2	0	0	0	0	0	-	-	-	-	-	-	-	-
Hypoactivity	5	5	5	1	0	1	0	0	0	-	-	-	-	-	-	-	-
Miosis	5	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
Ataxia	1	2	1	1	0	1	0	0	0	-	-	-	-	-	-	-	-
Yellow-stained genital region	0	0	0	2	1	2	2	2	0	-	-	-	-	-	-	-	-
Gasping	0	0	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	1	1	1	0	0	0	-	-	-	-	-	-	-	-
Respiratory congestion	0	0	0	1	0	0	0	0	0	-	-	-	-	-	-	-	-
Diarrhea	0	0	0	0	1	0	0	0	0	-	-	-	-	-	-	-	-
Tonic convulsions	0	0	0	0	0	1	0	2	0	-	-	-	-	-	-	-	-
Death	0	0	0	1A	1	0	1	1A	1	-	-	-	-	-	-	-	-

A - Found dead at p.m. mortality check.

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

PATHOLOGY

DOSE LEVEL: 0.50 g/kg of body weight

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C03081	M	4	-	No visible lesions.
C03080	M	4	-	Ventral cervical region and both mandibles cannibalized.
C03082	M	-	14	No visible lesions.
C03079	M	-	14	No visible lesions.
C03088	M	-	14	No visible lesions.
C03249	F	-	14	No visible lesions.
C03247	F	-	14	No visible lesions.
C03250	F	-	14	No visible lesions.
C03248	F	-	14	No visible lesions.
C03326	F	-	14	No visible lesions.

SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

PATHOLOGY (continued)

DOSE LEVEL: 5.0 g/kg of body weight

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C00499	M	4	-	Perineum stains - tan; stomach - glandular portion has dark brown focal areas (1 mm in diameter).
C00504	M	3	-	Perineum/perianal stains - yellow; nasal discharge - dark red and crusted.
C00503	M	2	-	Nasal and mouth areas - stained brown; stomach - glandular portion has dark brown areas (up to 5 x 1 mm).
C00501	M	1	-	Stomach - glandular portion has multiple, dark brown, pinpoint foci.
C00562	M	2	-	Stomach - glandular portion has dark brown areas (up to 2 x 1 mm).
C03035	F	2	-	No visible lesions.
C03034	F	4	-	Stomach - glandular portion has dark brown areas (up to 3 x 1 mm).
C03032	F	6	-	Stomach - glandular mucosa has dark brown areas (up to 4 x 1 mm).
C03029	F	1	-	No visible lesions.
C03038	F	5	-	Perineum stains - brown; paranasal discharge - red.

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SAMPLE NUMBER: 70905761

SAMPLE: T-4102

OECD ORAL SCREEN (CONTINUED)

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, Acute Oral Toxicity, adopted May 12, 1981.

2. OECD's Principles of Good Laboratory Practice, Annex 2, C(81)30 (Final).

3. NIH Publication No. 86-23 (revised 1985).

HLA LAB NO. 70905761
PATHOLOGY REPORT
Acute Oral Toxicity

Ten rats (five males, five females) from each of two dose levels (0.5 and 5.0 g/kg) were necropsied. The animals that died on test were refrigerated and necropsied within 24 hours, usually the same day. All surviving animals were euthanatized and necropsied at the termination of the study. The dose level, day of death, and gross observations recorded for each animal are on pages 8 and 9 of this report.

The most frequently recorded observations were in those animals that died on test. The mucosa of the stomach (glandular portion) of several animals had dark brown areas of variable size which were possibly treatment related. All other observations were considered related to postmortem change or incidental findings which were not related to treatment.

Thomas E. Palmer, PhD
Pathologist

1-25-88
Date

(1734mcs)

HLA No. 10905761

Personnel Signature Sheet
Acute Toxicology

Name	Job Title	Signature	Initials
Becky Beckwith	Sr. Lab. Animal Asst.	Becky Beckwith	BB
Steve Beloungy	Lab. Animal Asst.	Steve Beloungy	SB
Ken Bridges	Sr. Lab. Animal Asst.	Ken Bridges	KB
Pat Crary	Sr. Lab. Animal Asst.	Pat Crary	PC
Shane Eith	Lab Animal Asst.	Shane Eith	SE
Shawn Frazier	Lab Animal Asst.	Shawn Frazier	SF
Steven M. Glaza	Group Leader	Steven M. Glaza	SG
Molly Hahn	Admin. Clerk	Molly Hahn	MH
Kevin Hamilton	Lab. Animal Asst.	Kevin Hamilton	KH
Jeff Hicks	Sr. Lab. Animal Asst.	Jeff Hicks	JH
Calvin Horton	Group Leader	Calvin Horton	CH
Sharen L. Howery	Administrative Asst.	Sharen L. Howery	SLH
Gregory Johnson	Lab. Animal Tech.	Gregory Johnson	GJ
Paul Krebs	Lab. Animal Asst.	Paul Krebs	P.K.
Wayne Madison	Section Supervisor	Wayne A. Madison	WAM
Scott McConnell	Lab. Animal Tech.	Scott McConnell	SMc
Shelley McConnell	Lab. Animal Tech.	Shelley McConnell	SMc
Dawn Conant	Sr. Lab. Animal Asst.	Dawn Conant	DC
Don Mavis	Lab. Animal Asst.	Don Mavis	DM
Robin Olson	Lab. Animal Asst.	Robin Olson	RO
Patricia Padgham	Team Leader	Patricia Padgham	PP
Joseph J. Daun	Lab. Animal Asst.	Joseph J. Daun	JJD
Michael Patzka	Lab. Animal Asst.	Michael Patzka	MP
John Paulson	Sr. Lab. Animal Asst.	John Paulson	JW
Jane Polnow	Lab. Animal Tech.	Jane Polnow	JP
Dennis B. Steiner	Lab. Animal Tech.	Dennis B. Steiner	D.S.
Michael Thesing	Lab. Animal Asst.	Michael Thesing	MT
Paula G. Vangen	Administrative Asst.	Paula G. Vangen	PGV
Albert Olson	Manpower	Albert Olson	AO
Jim Jirschele	LTE	Jim Jirschele	JJ
Ben Haley	Sr. Lab Animal Asst.	Ben Haley	BH

HLA: 70905761

DOSE ADMINISTRATION/BODY WEIGHT/MORTALITY RECORD

Study Title: Acute Oral Toxicity

Test Material: T-4102 Vehicle: NA

Bulk Density: 1.06 (g/mL) Species/Strain: Albino Rat/Sprague-Dawley Source: Charles River Date Received: 10/12/87 ♂ 10/26/87 ♀

Dose Level: 0.50 (g/kg) Fasted Date/Time/Technician 11/5/87/3:30 p.m./ BH Room No. 5

Route of Administration: Oral Gavage

Dose Volume 0.47 (mL/kg) Sex: (Male)/Female Dose Time: 11:25Am

Animal No.	CO	3081	3082	3080	3079	3088
Prefasted Body Weight (g)		NA				
Fasted Body Weight (g)		248	263	284	280	283
Actual Dose (mL)		0.12	0.12	0.13	0.13	0.13
Day 7 Body Weight (g)		Found Dead 11-10-87 BH	263	Found Dead 11/10/87 BH	289	266
Day 14 Body Weight (g)		235	328	247	349	340
Dead Body Weight (g)						

Technician	1987 Date	Scale Used/KTRON
BH	11/6	NA
		→
BH	11/6	5228
BH	11/6	Verified by: M 11/6/87
BH	11/13	15019
BH	11/20	5228

Dose Volume 0.47 (mL/kg) Sex: Male/(Female) Dose Time: 11:30Am

Animal No.	CO	3249	3247	3250	3248	3326
Prefasted Body Weight (g)		NA				
Fasted Body Weight (g)		214	212	222	200	201
Actual Dose (mL)		0.10	0.10	0.10	0.09	0.09
Day 7 Body Weight (g)		195 BH	205 BH	218 BH	194 BH	188 BH
Day 14 Body Weight (g)		218	248	254	239	229
Dead Body Weight (g)						

Technician	1987 Date	Scale Used KTRON
BH	11/6	NA
		→
BH	11/6	5228
BH	11/6	Verified by: M 11/6/87
BH	11/13	15019
BH	11/20	5228

MORTALITY (NO. DIED/NO. DOSED)

Dose Level (g/kg)	Sex	Hours 0 - 4	Study Day 1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM	Total
0.5	♂	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5
0.5	♀	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	NA	0/5
Tech.		BH																											NA	NA	BH
Date 1987		11/6	11/7	11/7	11/8	11/8	11/9	11/9	11/10	11/10	11/11	11/11	11/12	11/12	11/13	11/13	11/14	11/14	11/15	11/15	11/16	11/16	11/17	11/17	11/18	11/18	11/19	11/19	11/20	NA	11/20

NA - Not Applicable

* - Dosage calculated, but not administered unused animal returned to stock

Reviewed By [signature] Date 12/16/87

CO05541

000542

HLA: 70905761

OBSERVATIONS (Individual)

Dose level: 0.5 g./kg. Test material: T-4102

(Male) / Female [Male circled]

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
CO 3081	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	–										
	Dead	–	–	–	–	–	–	–	✓										
CO 3082	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓②	✓	✓	–	–	–	–	–	–	–	–
	Hypersensitive to touch	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–
CO 3080	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓										
	Dead	–	–	–	–	–	–	–	✓①										
CO 3079	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓②	–	–	–	–	–	–	–	–	–	–
	Hypoactive	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–
CO 3088	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓②	–	–	–	–	–	–	–	–	–	–
	Hypoactive	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–	–
	hypersensitive to touch	–	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–
	intermittent clonic convulsions	–	–	–	–	–	–	–	–	–	③✓	–	–	–	–	–	–	–	–
	Deaths	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	Collected by	BA	BA	BA	BA	JP	SAM	BA	BA	SMC	BA	BA	BB	BB	BA	BA	BA	BA	BA
	Date 1987	11/6	11/6	11/6	11/6	11/7	11/8	11/9	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17	11/18	11/19	11/20

① Found dead 11am 11/10/87 BA
② Noticed aggressive behavior 3 hrs after AM check 11/10/87 BA

- Surviving animals submitted for terminal necropsy.
  Technician BA
  Date 11/20/87

- Surviving animals designated for sacrifice and discard.
  Technician NA
  Date NA

✓ Indicates condition exists
Sl Slight.
- Condition not evident.
* Found dead, P.M. check.
NA - NOT APPLICABLE 12-16-87

Reviewed by: [signature]
Date: 12-16-87

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HLA: 70905761

OBSERVATIONS (Individual)

Dose level: 0.5 g./kg. Test material: T-4102

Male/Female

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
CO 3249	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓②	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–	–
	Hypersensitive to touch	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–
CO 3247	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓②	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–	–
	Hypoactive	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–
	Ataxic	–	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–
	⑤ intermittent clonic convulsions	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–	–
CO 3250	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓②	–	–	–	–	–	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–	–
	Hypersensitive to touch	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	–	–	–	–	–
																		✓	
CO 3248	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓②	–	–	✓	✓	✓	✓	✓	✓	✓	✓
	aggressive behavior	–	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–	–	–
	⑤ intermittent clonic convulsions	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–	–
	hypersensitive to touch	–	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–
CO 3324	Appeared Normal	✓	✓	✓	✓	✓	✓	✓	✓②	–	–	–	–	–	–	–	–	–	–
	aggressive behavior	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–	–	–
	alopecia back region	–	–	–	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	hypersensitive to touch	–	–	–	–	–	–	–	–	–	✓	–	–	–	–	–	–	–	–
	Deaths																		
	Collected by	BH	BH	BH	BH	BH	BH	BH	BH	BH	BH	BH	BB	BB	BH	BH	BH	BH	BH
	Date 1987	11/6	11/6	11/6	11/6	11/7	11/8	11/9	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17	11/18	11/19	11/20

① Incorrect entry 11/10/87 BH

② Entry errors 11-11-87 SM

- Surviving animals submitted for terminal necropsy. Technician BH Date 11/20/87

- Surviving animals designated for sacrifice and discard. Technician NA Date NA

✓ Indicates condition exists
Sl Slight.
– Condition not evident.
* Found dead, P.M. check.
NA - NOT APPLICABLE 12-16-87 JM

Reviewed by: JM
Date: 12-16-87

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000543

HLA: 70905761

DOSE ADMINISTRATION/BODY WEIGHT/MORTALITY RECORD

Study Title: Acute Oral Toxicity

Test Material: T-4102 Vehicle: NA

Bulk Density: 1.06 (g/mL) Species/Strain: Albino Rat/Sprague-Dawley Source: Charles River Date Received: 9/8/87 ♂ 10/12/87 ♀

Dose Level: 5.0 (g/kg) Fasted Date/Time/Technician ~~10/25/87~~ 10/25/87 2:30 p.m./ BB Room No. 5

Route of Administration: Oral Gavage

Dose Volume ①4.72 6.94 (mL/kg)	Sex: Male/Female					Dose Time: 11:15 Am		Technician	Date 1987	Scale Used KTRON
Animal No. CO	0499	0504	0503	0501	0562			BH	10/26	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	274	262	260	227	251			BH	10/26	15019
Actual Dose (mL)	1.3	1.2	1.2	1.1	1.2			BB	10/26	Verified by: TH 10-26-87
Day 7 Body Weight (g)	Dead 10/30/87 BH	Dead 10/29/87 BH	Dead 10/28/87 BH	Dead 10/27/87 BH	Dead 10/28/87 BH					
Day 14 Body Weight (g)										
Dead Body Weight (g)	234	240	232	213	232					

Dose Volume ①4.72 6.94 (mL/kg)	Sex: Male/Female					Dose Time: 11:10 Am		Technician	Date 1987	Scale Used KTRON
Animal No. CO	3035	3034	3032	3029	3038			BH	10/26	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	213	220	221	214	211			BH	10/26	15019
Actual Dose (mL)	1.0	1.0	1.0	1.0	0.9 1.0			BB	10/26	Verified by: TH 10-26-87
Day 7 Body Weight (g)	Dead 10/28/87 BH	Dead 10/30/87 BH	DEAD 11/1/87 KB	Dead 10/27/87 BH	DEAD 10-31-87 KB					
Day 14 Body Weight (g)										
Dead Body Weight (g)	189	195	178	202	167					

MORTALITY (NO. DIED/NO. DOSED)

Dose Level (g/kg)	Sex	Hours 0 - 4	Study Day 1 AM	1 PM	2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM	11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM	Total
5.0	♂	0/5	0/5	1/5	4/5	0/5	1/5	0/5	1/5	2/5	0/5	1/5	1/5	NA																NA	5/5
5.0	♀	0/5	0/5	1/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	NA																NA	5/5
Tech.		BB	BH	BH	BH	BH	BH	BH	BH	BB	KB	KB	KB																	NA	BH
Date 1987		10/26	10/27	10/27	10/28	10/28	10/29	10/29	10/30	10/30	10/31	10/31	11/1																	NA	11/13

NA - Not Applicable
* - Dosage calculated, but not administered unused animal returned to stock

Reviewed By [signature] Date 10-16-87

①Entry Errors BB 10/26/87
②Entry Error Corrected late. BB 10/27/87

③ entry errors all animals dead on 10/30 AM. MO 11-1-87

C00544

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HLA: 70905761

OBSERVATIONS (Individual)

Dose level: 5.0g/Kg Test material: T-4102

(Male)/Female

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Co-0499	Appeared Normal	✓	-	-	-	-	-	-	-										
	hyperactive	—	✓	✓	✓	✓	✓	-	-										
	diarrhea	—	—	—	—	—	—	✓	-										
	convulsions (tonic)	—	—	—	—	—	—	✓	-										
	ataxic	—	—	—	—	—	—	✓	-										
	excessive salivation	—	—	—	—	—	—	✓	-										
	Dead								✓										
Co-0504	Appeared Normal	✓	-	-	-	-	-	-											
	hyperactive	—	✓	✓	✓	✓	✓	-											
	Miosis	—	✓	-	-	-	-	-											
	red stained face	—	—	—	—	✓	✓	-											
	yellow stained genital region	—	—	—	—	—	✓	-											
	pain reflex absent	—	—	—	—	—	✓	-											
	Dead	—	—	—	—	—	—	✓											
Co-0503	Appeared Normal	✓	-	-	-	-	-												
	hyperactive	—	✓	✓	✓	✓	-												
	Miosis	—	✓	-	-	-	-												
	red stained face	—	—	—	—	✓	-												
	Dead	—	—	—	—	—	✓												
Co-0501	Appeared Normal	✓	-	-	-	-													
	hyperactive	—	✓	✓	✓	✓													
	Ataxic	—	✓	✓	-	-													
	Miosis	—	✓	-	-	-													
	lacrimation	—	—	—	—	✓													
	red stained face	—	—	—	—	✓													
	Dead	—	—	—	—	✓*													
Co-0562	Appeared Normal	✓	-	-	-	-	-												
	Ataxic	—	✓	-	-	-	-												
	hyperactive	—	✓	✓	✓	✓	-												
	miosis	—	✓	-	-	-	-												
	lacrimation	—	—	—	—	✓	-												
	Dead	—	—	—	—	—	✓												
	Deaths	0	0	0	0	1	2	1	1										
	Collected by	BB	BB	BB	BB	BH	BH	BH	BH										
	Date 1987	10/26	10/26	10/26	10/26	10/27	10/28	10/29	10/30										

- Surviving animals submitted for terminal necropsy.
Technician NA
Date NA

- Surviving animals designated for sacrifice and discard.
Technician NA
Date NA

✓ Indicates condition exists
SI Slight.
- Condition not evident.
* Found dead, P.M. check.
NA-NOT APPLICABLE 12-16-87 DD

Reviewed by: DD
Date: 12-16-87

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GD05545

HLA: 70905761

OBSERVATIONS (Individual)

Dose level: 5.0g/Kg Test material: T-4102

Male/Female

Animal Number	Observations	Pre-dose	Hours 1	Hours 2.5	Hours 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Co- 3035	Appeared Normal	✓	–	–	–	–	–												
	hyperactive	–	✓	✓	✓	–	–												
	miosis	–	✓	–	–	–	–												
	yellow stained genitals	–	–	–	–	✓	–												
	Dead	–	–	–	–	–	✓												
Co- 3035① 3034	Appeared Normal	✓	–	–	–	✓	–	–	–										
	hyperactive	–	✓	✓	✓	–	–	✓	–										
	miosis	–	✓	–	–	–	–	–	–										
	red stained face	–	–	–	–	–	✓	✓	–										
	convulsions (tonic)	–	–	–	–	–	–	✓	–										
	ataxic	–	–	–	–	–	–	✓	–										
	Dead	–	–	–	–	–	–	–	✓										
Co- 3032	Appeared Normal	✓	–	–	–	✓	–	–	–	–	–								
	hyperactive	–	✓	✓	✓	–	–	–	–	–	–								
	miosis	–	✓	–	–	–	–	–	–	–	–								
	diarrhea	–	–	–	–	–	✓	–	–	–	–								
	yellow stained genital region	–	–	–	–	–	–	✓	✓	✓	–								
	tonic convulsions	–	–	–	–	–	–	–	–	✓	–								
	DEAD	–	–	–	–	–	–	–	–	–	✓								
Co- 3029	Appeared Normal	✓	–	–	–	DEAD													
	hyperactive	–	✓	✓	✓	✓													
	miosis	–	✓	–	–	–													
	Ataxic	–	–	✓	–	✓													
	gasping	–	–	–	–	✓													
	yellow stained genitals	–	–	–	–	✓													
	red stained face	–	–	–	–	✓													
Co- 3038	Appeared Normal	✓	–	–	–	–	–	–	–	–									
	Ataxic	–	✓	✓	✓	–	–	–	–	–									
	hyperactive	–	✓	✓	✓	–	–	–	–	–									
	miosis	–	✓	–	–	–	–	–	–	–									
	respiratory congestion	–	–	–	–	✓	–	–	–	–									
	yellow stained genital region	–	–	–	–	–	✓	✓	✓	✓									
	tonic convulsions	–	–	–	–	–	–	–	–	✓*									
	Deaths	0	0	0	0	1	1	0	1	0/1	1								
	Collected by	BB	BB	BB	BB	BH	BH	BH	BH	AD	KB								
	Date 1987	10/26	10/26	10/26	10/26	10/27	10/28	10/29	10/30	10/31	11/1								

①Entry Error BB 10/26/87

- Surviving animals submitted for terminal necropsy. Technician NA Date NA

- Surviving animals designated for sacrifice and discard. Technician NA Date NA

✓ Indicates condition exists
Sl Slight.
– Condition not evident.
* Found dead, P.M. check.
NA-NOT APPLICABLE 12-16 BB

Reviewed by: JPP
Date: 12-16-87

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HAZLETON LABORATORIES AMERICA, INC.
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

ROGER G. PERKINS
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 70905764
SAMPLE ENTERED: 09/25/87
REPORT PRINTED: 01/20/88

SAMPLE: T-4102

PURCHASE ORDER NUMBER: T837389-410 754

ENCLOSED: PRIMARY EYE IRRITATION/CORROSION STUDY IN RABBITS
(OECD GUIDELINES)

- o Key Personnel
- o Method
- o Summary
- o References
- o Raw Data Appendix

SIGNED: Steven M. Glaza
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

DATE 1-20-88

SAMPLE NUMBER: 70905764

SAMPLE: T-4102

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director

Calvin L. Horton
Group Leader
Support Services

Sharen L. Howery
Report Coordinator

Quality Assurance

Debra Curley Arndt
Manager

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Robert Salava
Senior Section Supervisor

Dennis Hoffman
Group Leader
Necropsy

Anne Mosher
Group Leader
Pathology Data

Laboratory Animal Veterinarian

Cindy J. Cary, DVM
Diplomate, ACLAM

SAMPLE NUMBER: 70905764

SAMPLE: T-4102

OECD EYE IRRITATION

Objective: To determine the level of ocular irritation produced following a single exposure of a test substance to one eye of albino rabbits according to the Organization for Economic Cooperation and Development's Guidelines for Testing Chemicals.

Test Material: T-4102

Physical Description:	Dark amber liquid
Purity and Stability:	Sponsor assumes responsibility for purity and stability determinations.
Storage and Retention:	The test material was stored at room temperature. Any unused material will be discarded according to HLA Standard Operating Procedure.
Safety Precautions:	Normal handling procedures were used according to HLA Standard Operating Procedure.

Test Animal: Young adult rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days. Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals". If variations from the prescribed environmental conditions existed, they were documented and considered to have no effect on the study outcome. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

Three acclimated animals, weighing from 2076 to 2155 g, were chosen at random for the test. The animals' eyes were examined within 24 hours prior to test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used. Test animals were identified by animal number and corresponding ear tag.

Reason for Species Selection: The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Preparation and Administration of Test Material: The sample was dosed as received. The pH was determined to be 9.1.

Treatment: Each rabbit received 0.1 ml of the liquid test material placed into the everted lower lid of one eye, with the contralateral eye serving as the untreated control. The upper and lower lids were gently held together for one second to prevent loss of material and then released. The eyes of the rabbits remained unflushed.

SAMPLE NUMBER: 70905764 PAGE 4

SAMPLE: T-4102

OECD EYE IRRITATION (CONTINUED)

Reason for Route of Administration: Historically, the route of choice based on the method of Draize.

Observations: The treated eyes were observed for ocular irritation at 1, 24, 48, 72 and 96 hours, and at 7 and 14 days after treatment.

At the 72-hour, 7- and 14-day readings, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize technique.

Animals were weighed just prior to test material administration. Body weights were taken again at weekly intervals throughout the study period or at death.

Pathology: At study termination surviving animals were euthanatized and discarded. The animal that died on study was subjected to a gross necropsy examination and abnormalities were recorded. After necropsy, the animal was discarded and no tissues were saved.

Statistical Methods: Other than average eye irritation scores, no other statistical method was performed.

Location of Raw Data and Final Report: The raw data and a copy of the final report will be retained in the archives of HLA.

SAMPLE NUMBER: 70905764

SAMPLE: T-4102

OECD EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 10/20/87

Date Test Started: 11/06/87 Date Test Completed: 11/20/87

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	3 Rabbit Mean 0.1 ml (Unwashed)
1 Hour:	24.0
24 Hours:	29.3
48 Hours:	21.3
72 Hours:	16.5**
96 Hours:	5.5**
7 Days:	1.0**
14 Days:	0.0**

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (3) at each observation period.
** Based on a two-animal mean.

SAMPLE NUMBER: 70905764 PAGE 6

SAMPLE: T-4102

OECD EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F20347	1 Hour	0	0	0	1	5	2	2	2	12
	24 Hours	1	3	15	1	5	2	2	1	10
	48 Hours	1	3	15	1	5	2	2	1	10
	72 Hours	1	2	10	1	5	2	2	1	10
	96 Hours	1	1	5	0	0	1	0	0	2
	7 Days	0	0	0	0	0	1	0	0	2
	14 Days	0	0	0	0	0	0	0	0	0
F20333	1 Hour	1	1	5	1	5	2	3	3	16
	24 Hours	1	2	10	1	5	2	3	2	14
	48 Hours	1	1	5	1	5	2	3	3	16
	72 Hours	0	0	0	0	0	2	1	1	8
	96 Hours	0	0	0	0	0	1	1	0	4
	7 Days	0	0	0	0	0	0	0	0	0
	14 Days	0	0	0	0	0	0	0	0	0
F20344*	1 Hour	1	2	10	1	5	2	3	2	14
	24 Hours	1	2	10	1	5	2	3	2	14
	48 Hours	0	0	0	0	0	2	1	1	8

Cornea
A = Degree of opacity
B = Area of involvement

Conjunctivae
A = Redness
B = Chemosis
C = Discharge

Table 2
Sodium Fluorescein Examination

Animal Number	Observation Period: Pre-initiation	72 Hours	7 Days	14 Days
F20347	NEG	POS (40%)	NEG	NEG
F20333	NEG	NEG	NEG	NEG
F20344	NEG	*	*	*

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

* Animal found dead at 72-hour observation.

SAMPLE NUMBER: 70905764 PAGE 7

SAMPLE: T-4102

OECD EYE IRRITATION (CONTINUED)

Comments:

A pain response (excessive pawing at the treated eye) was elicited from two animals immediately following instillation of the test material.

Blanching of the conjunctivae was seen in all three animals at 1, 24, and 48 hours, and in the remaining two animals at 72 hours.

Petite hemorrhaging of the conjunctivae was seen in two animals at 48 hours.

Corneal epithelial peeling was exhibited by two animals at 1 hour, by all three animals at 24 hours, by two animals at 48 hours, and by one animal at 72 and 96 hours.

Animal No. F20344 was found dead on Study Day 3. The gross necropsy revealed no visible lesions.

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 405, Acute Eye Irritation/Corrosion, adopted May 12, 1981.

2. Draize J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the United States, pp. 46-59 (1975).

3. 40 CFR 792.

4. NIH Publication No. 86-23 (Revised 1985).

HLA No. 70905764

Personnel Signature Sheet
Acute Toxicology

Name	Job Title	Signature	Initials
Becky Beckwith	Sr. Lab. Animal Asst.	Becky Beckwith	BB
Steve Beloungy	Lab. Animal Asst.	Steve D Beloungy	SB
Ken Bridges	Sr. Lab. Animal Asst.	Ken Bridges	KB
Pat Crary	Sr. Lab. Animal Asst.	Pat Crary	PC
Shane Eith	Lab Animal Asst.	Shane Eith	SE
Shawn Frazier	Lab Animal Asst.	Shawn Frazier	SF
Steven M. Glaza	Group Leader	Steven M. Glaza	SG
Molly Hahn	Admin. Clerk	Molly Hahn	MH
Kevin Hamilton	Lab. Animal Asst.	Kevin Hamilton	KH
Jeff Hicks	Sr. Lab. Animal Asst.	Jeff Hicks	JH
Calvin Horton	Group Leader	Calvin Horton	CH
Sharen L. Howery	Administrative Asst.	Sharen L. Howery	SLH
Gregory Johnson	Lab. Animal Tech.	Gregory Johnson	GJ
Paul Krebs	Lab. Animal Asst.	Paul P Krebs	P.K.
Wayne Madison	Section Supervisor	Wayne A. Madison	WAM
Scott McConnell	Lab. Animal Tech.	Scott McConnell	SMM
Shelley McConnell	Lab. Animal Tech.	Shelley McConnell	SMC
Dawn Conant	Sr. Lab. Animal Asst.	Dawn Conant	DC
Don Mavis	Lab. Animal Asst.	Don Mavis	DM
Robin Olson	Lab. Animal Asst.	Robin Olson	RO
Patricia Padgham	Team Leader	Patricia Padgham	PP
Joseph J. Daun	Lab. Animal Asst.	Joseph J Daun	JD
Michael Patzka	Lab. Animal Asst.	Michael Patzka	MP
John Paulson	Sr. Lab. Animal Asst.	John Paulson	JW
Jane Polnow	Lab. Animal Tech.	Jane Polnow	JP
Dennis B. Steiner	Lab. Animal Tech.	Dennis B. Steiner	DS
Michael Thesing	Lab. Animal Asst.	Michael Thesing	MT
Paula G. Vangen	Administrative Asst.	Paula G Vangen	PGV
Albert Olson	Manpower	Albert J. Olson	AO
Jim Jirschele	LTE	Jim Jirschele	JJ
Ben Haley	Sr. Lab Animal Asst.	Ben Haley	BH
Eileen M. McConnell	Admin. Clerk	Eileen M McConnell	EMM
Annette R. Turner	Manpower	Annette R Turner	ART

HLA: 70905764

BODY WEIGHT/DOSE RECORD

Study title: Primary Eye Irritation
Test material: T-4102
Physical description: DARK AMBER LIQUID
pH result: 9.1 with Hanna Meter No. 6890
Dose: 0.1 mL/ NA g (NA mL equivalent)
✓ dosed with a 1-mL disposable syringe/ NA each dose
individually drawn up with a NA-µL Hamilton Syringe

Date animals received: 10-20-87
Species/strain: Rabbit/New Zealand White
Source: Hazleton Research Products, Inc.

Room No: 161-E

Review of folder preparation by: NP Date: 11-6-87

Animal No.	Group	Sex	Initial SF*	Pain Response Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
F20347	1	♀	NEG	N	2135	2358	2461	
F20333	1	♀	NEG	E	2155	2148 BB	2316	
F20344	1	♀	NEG	E	2076	FOUND DEAD 11/9/87 KB 1855g		
Technician		JH	JH	KB/g.w.	KB	BB	BB	
Date	1987	11-5	11-5	11/6	11/6	11/13	11/20	
Scale Used				KTRON	15019	1348	15019	

All animals appeared normal just prior to dosing.
Technician: KB Date: 11/6/87

* - Sodium fluorescein examination.
NEG - Negative.
POS - Positive.
NA - Not applicable.
U - Unable to determine pH.
V - Vocalization.
E - Excessive pawing of treated eye.
N - None

NA Dosed directly on the cornea. The eyelids were released immediately without forced blinking or manipulation.

✓ Dosed in the conjunctival sac. The upper and lower lids were gently held together for one second.

Time of dosing: 2:30 PM
Technician KB/g.w. Date: 11/6/87
Time of first observation: 3:30 PM
Technician: NP Date: 11/6/87

Surviving animals designated for sacrifice and discard. Technician KB Date 11/21/87

Reviewed by: NP Date: 12-8-87

(00261/vmt)

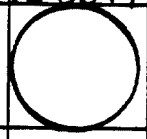
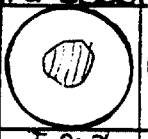
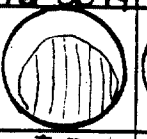
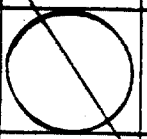
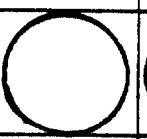
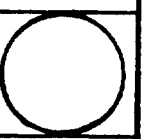
HLA: 709057604

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T.4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: 1 hour

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head						
Cornea - Opacity	0	J=20% 1	J=50% 1			
Area	0	1	2			
Iris	1 I	1 I	1 I			
Conjunctivae - Redness	2 B	2 B	2 B			
Chemosis	2	3	3			
Discharge	2 C	3 C	2 C			
Sodium fluorescein exam	NA	NA	NA			

Technician: KB/MP

Date: 11/6/87

EYE IRRITATION SCORE: 24.0
Calculated By: [illegible] Date 11-9-87
Verified By: MP Date 11-13-87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: MP Date: 12-8-87

(00261/vmt)

HLA: 70905764

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T-4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: 24 hours

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head	 J=75%	 J=50%	 J=35%			
Cornea - Opacity	1	1	1			
Area	3	2	2			
Iris	1 I	1 I	1 I			
Conjunctivae - Redness	2 B	2 B	2 B			
Chemosis	2	3	3			
Discharge	1 C	2 D	2 D			
Sodium fluorescein exam	NA	NA	NA			

Technician: [signature]

Date: 11-7-87

EYE IRRITATION SCORE: 29.3
Calculated By: [signature] Date 11-9-87
Verified By: [signature] Date 11-13-87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: [signature] Date: 12-8-87

(00261/vmt)

HLA: 70905764

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T-4102
Test eye: Right

UAWashed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: 48 hours

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head	 J: 20%	 J=15%				
Cornea - Opacity	1	1	0			
Area	3	1	0			
Iris	1I	1I	0			
Conjunctivae - Redness	2AB	2AB	2B			
Chemosis	2	3	1			
Discharge	1D	3C	1C			
Sodium fluorescein exam	NA	NA	NA			

Technician: Sam

Date: 11-8-87

EYE IRRITATION SCORE: 21.3
Calculated By: SH Date 11-9-87
Verified By: M Date 11-13-87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: M Date: 12-8-87

(00261/vmt)

HLA: 70905764

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T.4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: 72 hours

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head						
Cornea - Opacity	I=40% 1	0	NA			
Area	2	0				
Iris	1 I	0				
Conjunctivae - Redness	2 B	2 B				
Chemosis	2	1				
Discharge	1 C	1 D				
Sodium fluorescein exam	Pos 40%	Neg				

EYE IRRITATION SCORE: 16.5 (2-ANIMAL MEAN)
Calculated By: [illegible] Date 11-9-87
Verified By: [illegible] Date 11-13-87

Technician: [illegible]

Date: 11-9-87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: [illegible] Date: 12-8-87

(00261/vmt)

HLA: 70905764

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T-4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: 96 hours

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head						
Cornea - Opacity	1 J=1½	0	NA			
Area	1	0	↓			
Iris	0	0				
Conjunctivae - Redness	1	1				
Chemosis	0	1				
Discharge	0	0				
Sodium fluorescein exam	NA	NA	↓			

Technician: MD/BA

Date: 11-10-87

EYE IRRITATION SCORE: 5.5 (2-animal me
Calculated By: SV Date 11-12-87
Verified By: MP Date 11-13-87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: MP Date: 12-8-87

(00261/vmt)

HLA: 70905764

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T-4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: Day 7

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head	○	○	○	○	○	○
Cornea - Opacity	0	0	NA			
Area	0	0	↓			
Iris	0	0				
Conjunctivae - Redness	1	0				
Chemosis	0	0				
Discharge	0	0				
Sodium fluorescein exam	Neg	Neg	↓			

EYE IRRITATION SCORE: 1.0 (2-animal mean)
Calculated By: [illegible] Date 11-13-87
Verified By: [illegible] Date 11-16-87

Technician: BB
Date: 11/13/87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: [illegible] Date: 12-8-87

(00261/vmt)

HLA: 709057644

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
Test material: T.4102
Test eye: Right

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

Observation Period: Day 14

Animal No.	F2-0347	F2-0333	F2-0344			
Location of corneal lesions Tail ----- Head	○	○	○			
Cornea - Opacity	0	0	NA			
Area	0	0	↓			
Iris	0	0				
Conjunctivae - Redness	0	0				
Chemosis	0	0				
Discharge	0	0				
Sodium fluorescein exam	Neg	NEG	↓			

EYE IRRITATION SCORE: 0.0 (2-animal me
Calculated By: [illegible] Date 11-20-87
Verified By: M Date 12-8-87

Technician: BB
Date: 11/20/87

NA - Not applicable.
A - Petite hemorrhaging.
B - Blanching.
C - Clear discharge.
D - Purulent discharge.
E - Hair loss around the eye.
F - Necrotic areas.
G - Unable to visualize due to severe swelling.
H - No reaction to light.
I - Injected.
J - Corneal epithelial damage, peeling.
K - Corneal epithelial damage, piling.
L - Corneal epithelial damage, pitting.
M - Hypopyon.
N - Corneal neovascularization.
P - Pannus.
R - Unable to visualize due to severe opacity.
S - Granulation scar tissue.
POS - Positive stain retention.
NEG - Negative stain retention.

Reviewed by: M Date: 12-8-87

(00261/vmt)

HLA: 70905764

SCALE FOR SCORING OCULAR LESIONS
(DRAIZE[1] TECHNIQUE)

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one-quarter, but less than half	2
Greater than half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

A x B x 5 Total maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

A x 5 Total maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed	4*

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

* Indicates positive effect. (FHSA Interpretation)

[1] Draize, J. H., "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the United States, pp. 46-59 (1975).

(00261/vmt)

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FINAL REPORT

ROGER G. PERKINS
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 7090576
SAMPLE ENTERED: 09/25/8
REPORT PRINTED: 01/21/8

SAMPLE: T-4102

PURCHASE ORDER NUMBER: T837389-410 754

ENCLOSED: PRIMARY DERMAL IRRITATION/CORROSION STUDY IN RABBITS
(OECD GUIDELINES)

- Key Personnel
- Method
- Summary
- References
- Raw Data Appendix

SIGNED: Steven M. Glaza
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

DATE 1-21-88

SAMPLE NUMBER: 70905763

SAMPLE: T-4102

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director

Calvin L. Horton
Group Leader
Support Services

Sharen L. Howery
Report Coordinator

Quality Assurance

Debra Curley Arndt
Manager

Laboratory Animal Veterinarian

Cindy J. Cary, DVM
Diplomate, ACLAM

SAMPLE NUMBER: 70905763

SAMPLE: T-4102

OECD DERMAL IRRITATION

Objective: To determine the relative level of primary skin irritation/corrosion of a test substance on rabbits under semioccluded conditions according to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals.

Test Material: T-4102

Physical Description:	Dark amber liquid
Purity and Stability:	Sponsor assumes responsibility for purity and stability determinations.
Storage and Retention:	The test material was stored at room temperature. Any unused material will be discarded according to HLA Standard Operating Procedure.
Safety Precautions:	Normal handling procedures were used according to HLA Standard Operating Procedure.

Test Animal: Young adult rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days. Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals". If variations from the prescribed environmental conditions existed, they were documented and considered to have no effect on the study outcome. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

Three acclimated animals, weighing from 2202 to 2325 g, were chosen at random for the test, treated, and maintained during the observation period as specified for the acclimation period. Test animals were identified by animal number and corresponding ear tag. Approximately twenty-four hours before treatment the hair was clipped from the back of each animal.

Reason for Species Selection: Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

Preparation and Administration of Test Material: The sample was dosed as received. The pH was determined to be 9.1.

SAMPLE NUMBER: 70905763

SAMPLE: T-4102

OECD DERMAL IRRITATION (CONTINUED)

Treatment: The test material was applied to the intact skin of each rabbit in the amount of 0.5 ml. The treated area was covered with a 2.5 x 2.5-cm gauze patch secured with paper tape and overwrapped with Saran Wrap and Elastoplast tape to provide a semiocclusive dressing. Collars were applied to restrain the test animals for the 4-hour exposure period.

Reason for Route of Administration: Historically, the route of choice based on the method of Draize.

Observations: After the exposure period, the patches were removed. The test sites were washed using lukewarm tap water and disposable paper towels. The test material was removed from the test sites as thoroughly as possible without irritating the skin. Thirty minutes following removal of the test material, the degree of erythema and edema was read according to the Draize technique. Subsequent examinations were made at 24, 48 and 72 hours after patch removal.

Individual body weights were taken just prior to study initiation.

Pathology: At study termination all animals were euthanatized and discarded.

Statistical Methods: Other than average dermal irritation scores, no other statistical method was performed.

Location of Raw Data and Final Report: The raw data and a copy of the final report will be retained in the archives of HLA.

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SAMPLE NUMBER: 70905763

SAMPLE: T-4102

OECD DERMAL IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 10/20/87

Date Test Started: 11/02/87 Date Test Completed: 11/05/87

INDIVIDUAL DERMAL IRRITATION SCORES

Animal Number	Sex	Erythema Hours 4	24	48	72	Edema Hours 4	24	48	72
F20309	M	1	1	1	1	1	0	1	0
F20349	F	0	0	0	0	0	0	0	0
F20311	M	0	1	1	1	0	0	0	0
Mean		0.3	0.7	0.7	0.7	0.3	0.0	0.3	0.0

PRIMARY DERMAL IRRITATION SCORES *

Observation Period	3 Rabbit Mean
4 Hours:	0.7
24 Hours:	0.7
48 Hours:	1.0
72 Hours:	0.7

* The Primary Dermal Irritation Score is the total dermal irritation score for all the animals (erythema and edema) divided by the number of test sites (3) at each observation period.

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SAMPLE NUMBER: 70905763

SAMPLE: T-4102

OECD DERMAL IRRITATION (CONTINUED)

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 404, Acute Dermal Irritation/ Corrosion, adopted May 12, 1981.

2. OECD's Principles of Good Laboratory Practice, Annex 2, C(81)30 (Final)

3. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

4. NIH Publication No. 86-23 (revised 1985).

HLA No. 70905763

Personnel Signature Sheet
Acute Toxicology

Name	Job Title	Signature	Initials
Becky Beckwith	Sr. Lab. Animal Asst.	Becky Beckwith	BB
Steve Beloungy	Lab. Animal Asst.	Steve D Beloungy	SB
Ken Bridges	Sr. Lab. Animal Asst.	Ken Bridges	KB
Pat Crary	Sr. Lab. Animal Asst.	Pat Crary	PC
Shane Eith	Lab Animal Asst.	Shane Eith	SE
Shawn Frazier	Lab Animal Asst.	Shawn Frazier	SF
Steven M. Glaza	Group Leader	Steven M. Glaza	SG
Molly Hahn	Admin. Clerk	Molly Hahn	MH
Kevin Hamilton	Lab. Animal Asst.	Kevin Hamilton	KH
Jeff Hicks	Sr. Lab. Animal Asst.	Jeff Hicks	JH
Calvin Horton	Group Leader	Calvin D. Horton	CH
Sharen L. Howery	Administrative Asst.	Sharen L. Howery	SLH
Gregory Johnson	Lab. Animal Tech.	Gregory Johnson	GJ
Paul Krebs	Lab. Animal Asst.	Paul Krebs	P.K.
Wayne Madison	Section Supervisor	Wayne A. Madison	WAM
Scott McConnell	Lab. Animal Tech.	Scott McConnell	SMc
Shelley McConnell	Lab. Animal Tech.	Shelley McConnell	SMc
Dawn Conant	Sr. Lab. Animal Asst.	Dawn Conant	DC
Don Navis	Lab. Animal Asst.	Don Navis	DN
Robin Olson	Lab. Animal Asst.	Robin Olson	RO
Patricia Padgham	Team Leader	Patricia Padgham	PP
Joseph J. Daun	Lab. Animal Asst.	Joseph J. Daun	JD
Michael Patzka	Lab. Animal Asst.	Michael Patzka	MP
John Paulson	Sr. Lab. Animal Asst.	John Paulson	JW
Jane Polnow	Lab. Animal Tech.	Jane Polnow	JP
Dennis B. Steiner	Lab. Animal Tech.	Dennis B. Steiner	DS
Michael Thesing	Lab. Animal Asst.	Michael Thesing	MT
Paula G. Vangen	Administrative Asst.	Paula G. Vangen	PGV
Albert Olson	Manpower	Albert J. Olson	AO
Jim Jirschele	LTE	Jim Jirschele	JJ
Ben Haley	Sr. Lab Animal Asst.	Ben Haley	BH
Eileen M. McConnell	Admin. Clerk	Eileen M. McConnell	EMM
Annette R. Turner	Manpower	Annette R. Turner	ART

HLA: 70905763

DERMAL IRRITATION/BODY WEIGHT RECORD

(4-Hour Exposure)

Study Title: Primary Dermal Irritation
Test Material: T-4102
Physical Description: amber liquid (dark) pH Result: 9.1 with Hanna Meter No. 6890
Dose: 0.5 mL Per Site NA Moistened with 0.9% Saline
Date Animals Received: 10-20-87 Source/Strain/Species: Hazleton Research Products/New Zealand White/Rabbit Room Number: 161
Technician/Date Animals Clipped: KB / 11/1/87 Initiated by: M Date: 11-2-87
Skin Preparation: ✓ Intact NA Abraded (with a clipper blade) Reviewed by: [illegible] Date: 11/2/87

Animal Number/Sex	F2-0309 ♂	F2-0349 ♀	F2-0311 ♂				Technician	Recorded by	1987 Date	Scale used KTROD
Initial Body Weight (g)	2325	2202	2238				KB	KB	11/2	15019
7-Day Body Weight (g)										
14-Day Body Weight (g)										
21-Day Body Weight (g)										
Observation Period										Dermal Irritation Score
4 Hours Erythema	1	0	0				M	JH	11-2	✓ slw 11-9-87
4 Hours Edema	1	0	0							0.7 M 11-5-87
24 Hours Erythema	1	0	1				M	M	11-3	✓ slw 11-9-87
24 Hours Edema	0	0	0							0.7 M 11-5-87
48 Hours Erythema	1	0	1				[illegible]	JH	11-4	✓ slw 11-9-87
48 Hours Edema	1	0	0							1.0 M 11-5-87
72 Hours Erythema	1	0	1				KB	KB	11/5	✓ slw 11-9-87
72 Hours Edema	0	0	0							0.7 M 11-5-87
96 Hours Erythema										
96 Hours Edema										
7 Days Erythema										
7 Days Edema										
14 Days Erythema										
14 Days Edema										
21 Days Erythema										
21 Days Edema										

NA = Not applicable.
A = Subcutaneous hemorrhage.
B = Blanching.
N = Possible necrotic area.
U = Unable to determine pH.

All animals appeared normal just prior to dosing. Technician M Date 11-2-87
Surviving animals designated for sacrifice and discard. Technician [illegible] Date 11-6-87

Data reviewed by: [illegible] Date: 11-6-87

(10/'61/vmt)

① WRONG DATE ENTERED KB 11/2/87

TLS009

HLA: 70905763

PRIMARY DERMAL IRRITATION SCORING SCALE (DRAIZE[1] TECHNIQUE)

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

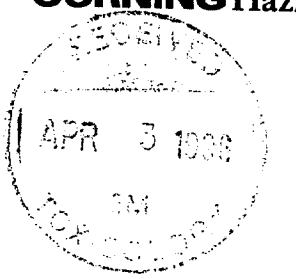
(2) Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

[1] Draize, J. H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal-Toxicity." Association of Food and Drug and Drug Officials of the U.S., pp. 46-59 (1975).

(00261/vmt)

CORNING Hazleton



MUTAGENICITY TEST WITH

T-6357

IN THE *SALMONELLA - ESCHERICHIA COLI*/MAMMALIAN-MICROSOME REVERSE MUTATION ASSAY

FINAL REPORT

AUTHOR

Timothy E. Lawlor, M.A.

PERFORMING LABORATORY

Corning Hazleton Inc. (CHV)
9200 Leesburg Pike
Vienna, Virginia 22182

LABORATORY PROJECT ID

CHV Study No.: 17387-0-409

SUBMITTED TO

3M
Building 220-2E-02 3M Center
St. Paul, MN 55144-1000

STUDY COMPLETION DATE

April 1, 1996

QUALITY ASSURANCE STATEMENT

STUDY TITLE: *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay

ASSAY NO.: 17387-0-409

PROTOCOL NO.: 409, Edition 4

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the Standard Operating Procedures of the Quality Assurance Unit and according to the general requirements of the appropriate Good Laboratory Practice regulations. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspection/Date	Findings Reported	Auditor
Characterization of Tester Strains - 02/14/96	02/14/96	C. Orantes
Draft Report Review - 03/16/96	03/18/96	S. Ballenger
Final Report Review - 04/01/96	04/01/96	S. Ballenger

Quality Assurance Unit — 4/1/96 Date Released

STUDY COMPLIANCE AND CERTIFICATION

The study was conducted in compliance with the Good Laboratory Practice regulations as set forth by the Food and Drug Administration (FDA) in Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978, (effective June 20, 1979) with any applicable amendments. There were no deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria.

Study Director:

Timothy E. Lawlor, M.A.
Bacterial Mutagenesis
Genetic and Cellular Toxicology

4.1.96
Study Completion Date

TABLE OF CONTENTS

SECTION I. SUMMARY

INTRODUCTION AND CONCLUSIONS

SUMMARY

A. Introduction

At the request of 3M, Corning Hazleton Inc. investigated **T-6357** for mutagenic activity in the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay. This assay evaluated the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains and at the tryptophan locus in an *Escherichia coli* tester strain both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor™-induced rat liver (S9).

The doses tested in the mutagenicity assay were selected based on the results of a dose rangefinding study using tester strains TA100 and WP2*uvr*A and ten doses of test article ranging from 5,000 to 6.67 µg per plate, one plate per dose, both in the presence and absence of S9 mix.

The tester strains used in the mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* tester strain WP2*uvr*A. The assay was conducted with five doses of test article in both the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per dose. The doses tested were 5,000, 3,330, 1,000, 333, and 100 µg per plate in both the presence and absence of S9 mix.

B. Conclusions

The results of the *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay indicate that, under the conditions of this study, 3M's test article, **T-6357**, did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9).

SECTION II. STUDY INFORMATION

STUDY INFORMATION

A. Sponsor: **3M**

B. Test Article: **T-6357**

1. Physical Description: **clear amber liquid**

2. Date Received: **01/16/96**

C. Type of Assay: *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay

1. Protocol Number: CHV Protocol 409, Edition 4

2. CHV Study Number: 17387-0-409

D. Study Dates

1. Study Initiation Date: **01/30/96**

2. Experimental Start Date: **02/04/96**

3. Experimental Termination Date: **02/21/96**

E. Study Supervisory Personnel

Study Director: Timothy E. Lawlor, M.A.

Laboratory Supervisor: Michael S. Mecchi, B.S.

SECTION III. MATERIALS AND METHODS

MATERIALS AND METHODS

The experimental materials, methods and procedures are based on those described by Ames *et al* (1975) and Green and Muriel (1976).

MATERIALS

A. Tester Strains

1. *Salmonella typhimurium*

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535, and TA1537 as described by Ames *et al* (1975). The specific genotypes of these strains are shown in Table I.

TABLE I. TESTER STRAIN GENOTYPES

Histidine Mutation			Additional Mutations		
*his*G46	*his*C3076	*his*D3052	LPS	Repair	R Factor
TA1535	TA1537		*rfa*	*uvr*B	-
TA100		TA98	*rfa*	*uvr*B	+R

In addition to a mutation in the histidine operon, the tester strains contain two additional mutations which enhance their sensitivity to some mutagenic compounds. The *rfa* wall mutation results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems (i.e. benzo(a)pyrene) that would otherwise be excluded by a normal intact cell wall.

The second mutation, a deletion of the *uvr*B gene, results in a deficient DNA excision repair system which greatly enhances the sensitivity of these strains to some mutagens. Since the *uvr*B deletion extends through the *bio* gene, all of the tester strains containing this deletion also require the vitamin biotin for growth.

Strains TA98 and TA100 also contain the R-factor plasmid, pKM101, which further increases the sensitivity of these strains to some mutagens. The mechanism by which this plasmid increases sensitivity to mutagens has been suggested to be by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. TA1535 is reverted by base substitution mutagens and TA100 is reverted by mutagens which cause both frameshifts and base substitutions.

2. *Escherichia coli*

The tester strain used was the tryptophan auxotroph WP2*uvr*A as described by Green and Muriel (1976).

In addition to a mutation in the tryptophan operon, the tester strain contains a *uvr*A DNA repair deficiency which enhances its sensitivity to some mutagenic compounds. This deficiency allows the strain to show enhanced mutability since the *uvr*A repair system would normally act to remove the damaged part of the DNA molecule and accurately repair it afterwards.

Tester strain WP2*uvr*A is reverted from tryptophan dependence (auxotrophy) to tryptophan independence (prototrophy) by base substitution mutagens.

3. Source of Tester Strains

a. *Salmonella typhimurium*

The tester strains in use at CHV were received directly from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley.

b. *Escherichia coli*

The tester strain, WP2*uvr*A, in use at CHV was received from the National Collection of Industrial Bacteria, Torrey Research Station, Scotland (United Kingdom).

4. Storage of the Tester Strains

a. Frozen Permanent Stocks

Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 ml/ml of culture) and freezing small aliquots (0.5-1.5 ml) at ≤- 70°C.

b. Master Plates

Master plates were prepared by streaking each tester strain from a frozen permanent stock onto minimal agar appropriately supplemented with 1) for *Salmonella typhimurium*, an excess of histidine, and biotin, and for tester strains TA 98 and TA100, ampicillin (25 µg/ml), to ensure the stable maintenance of the pKM101 plasmid; and 2) for *Escherichia coli*, an excess of tryptophan. Tester strain master plates were stored at 5 ± 3°C.

5. Preparation of Overnight Cultures

a. Inoculation

Overnight cultures for use in all testing procedures were inoculated by transferring a colony from the appropriate master plate to a flask containing culture medium. Inoculated flasks were placed in a shaker/incubator which was programmed to begin operation (shaking, 125 ± 25 rpm; incubation, 37 ± 2°C) so that the overnight cultures were in log phase or late log phase when turbidity monitoring began.

b. Harvest

To ensure that cultures were harvested in late log phase, the length of incubation was determined by spectrophotometric monitoring of culture turbidity. Cultures were harvested once a predetermined turbidity was reached as determined by a percent transmittance (%T) reading on a spectrophotometer. This target turbidity ensures that cultures have reached a density of at least 0.5 X 10^9 cells per ml and that the cultures have not overgrown. Overgrown (stationary) cultures may exhibit decreased sensitivity to some mutagens. Cultures were removed from incubation when the target %T was reached and were placed at 5 ± 3°C.

6. Confirmation of Tester Strain Genotypes

Tester strain cultures were checked for the following genetic markers on the day of their use in the mutagenicity assay:

a. *Salmonella typhimurium*

1) *rfa* Wall Mutation

The presence of the *rfa* wall mutation was confirmed by demonstration of the sensitivity of the culture to crystal violet. An aliquot of an overnight

culture of each strain was overlaid onto plates containing selective media and an antibiotic sensitivity disk containing 10 µg of crystal violet was added. Sensitivity was demonstrated by inhibition of bacterial growth in a zone immediately surrounding the disk.

2) pKM101 Plasmid R-factor

The presence of the pKM101 plasmid was confirmed for tester strains TA98 and TA100 by demonstration of resistance to ampicillin. An aliquot of an overnight culture of each strain was overlaid onto plates containing selective media and an antibiotic sensitivity disk containing 10 µg of ampicillin was added. Resistance was demonstrated by bacterial growth in the zone immediately surrounding the disk.

3) Characteristic Number of Spontaneous Revertants

The mean number of spontaneous revertants per plate in the vehicle controls that are characteristic of the respective strains were demonstrated by plating 100 µl aliquots of the culture along with the appropriate vehicle on selective media.

b. *Escherichia coli*

1) Characteristic Number of Spontaneous Revertants

The mean number of spontaneous revertants per plate in the vehicle controls that are characteristic of the respective strains were demonstrated by plating 100 µl aliquots of the WP2*uvr*A culture along with the appropriate vehicle on selective media.

7. Tester Strain Media

a. Culturing Broth

The broth used to grow overnight cultures of the tester strains was Vogel-Bonner salt solution (Vogel and Bonner, 1956) supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2 (dry powder).

b. Agar Plates

Bottom agar (25 ml per 15 x 100 mm petri dish) was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956), supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose.

c. Overlay Agar for Selection of Revertants

Overlay (top) agar was prepared with 0.7% agar (w/v) and 0.5% NaCl (w/v) and was supplemented with 10 ml of 1) 0.5 mM histidine/biotin solution per 100 ml agar for selection of histidine revertants, or 2) 0.5 mM tryptophan solution per 100 ml of agar for selection of tryptophan revertants. When S9 mix was required, 2.0 ml of the supplemented top agar was used in the overlay. However, when S9 mix was not required, water was added to the supplemented top agar (0.5 ml of water per 2 ml of supplemented top agar) and the resulting 2.5 ml of diluted supplemented top agar was used for the overlay. This dilution ensured that the final top agar and amino acid supplement concentrations remained the same both in the presence and absence of S9 mix.

B. Liver Microsomal Enzyme Reaction Mixture (S9 Mix)

1. S9 Homogenate

Liver microsomal enzymes (S9 homogenate) were purchased from Molecular Toxicology, Inc., Annapolis, MD 21401, Batch 0623 (42.4 mg of protein per ml). The homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 (200 mg per ml in corn oil) at 500 mg/kg as described by Ames *et al*, 1975.

2. S9 Mix

The S9 mix was prepared immediately prior to its use in any experimental procedure. The S9 mix contained the components indicated in Table II.

TABLE II. S9 MIX COMPONENTS

H_2O	0.70 ml
1M NaH_2PO_4/Na_2HPO_4, pH7.4	0.10 ml
0.25M Glucose-6-phosphate	0.02 ml
0.10M NADP	0.04 ml
0.825M KCl/0.2M $MgCl_2$	0.04 ml
S9 Homogenate	0.10 ml
	1.00 ml

C. Controls

1. Vehicle Controls

Vehicle controls were plated for all tester strains both in the presence and absence of S9 mix. The vehicle control was plated, using a 50 µl aliquot of vehicle (equal to the

maximum aliquot of test article dilution plated), along with a 100 µl aliquot of the appropriate tester strain and a 500 µl aliquot of S9 mix (when necessary), on selective agar.

2. Positive Controls

The combinations of positive controls, activation condition and tester strains plated concurrently with the assay are indicated in Table III.

TABLE III. POSITIVE CONTROLS

Tester Strain	S9 Mix	Positive Control	Conc per plate
TA98	+	2-aminoanthracene	2.5 µg
TA98	-	2-nitrofluorene	1.0 µg
TA100	+	2-aminoanthracene	2.5 µg
TA100	-	sodium azide	2.0 µg
TA1535	+	2-aminoanthracene	2.5 µg
TA1535	-	sodium azide	2.0 µg
TA1537	+	2-aminoanthracene	2.5 µg
TA1537	-	ICR-191	2.0 µg
WP2*uvr*A	+	2-aminoanthracene	25.0 µg
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1.0 µg

a. Source and Grade of Positive Control Articles

2-aminoanthracene (CAS #613-13-8), Sigma Chemical Co., purity ≥ 97.5%; 2-nitrofluorene (CAS #607-57-8), Aldrich Chemical Co., purity 98%; sodium azide (CAS #26628-22-8), Sigma Chemical Co., purity >98%; ICR-191 (CAS #1707-45-0), Polysciences Inc., purity >95%; 4-nitroquinoline-N-oxide (CAS #56-57-5), Sigma Chemical Co., purity >99%.

3. Sterility Controls

a. Test Article

The most concentrated test article dilution was checked for sterility by plating a 50 µl aliquot (the same volume used in the assay) on selective agar.

b. S9 Mix

The S9 mix was checked for sterility by plating 0.5 ml on selective agar.

METHODS

A. Dose Rangefinding Study

The growth inhibitory effect (cytotoxicity) of the test article to the test system was determined in order to allow the selection of appropriate doses to be tested in the mutagenicity assay.

1. Design

The dose rangefinding study was performed using tester strains TA100 and WP2*uvr*A both in the presence and absence of S9 mix. Ten doses of test article were tested at one plate per dose. The test article was checked for cytotoxicity up to a maximum concentration of 5 mg per plate.

a. Rationale

The cytotoxicity of the test article observed on tester strain TA100 is generally representative of that observed on the other tester strains and because of TA100's comparatively high number of spontaneous revertants per plate, gradations of cytotoxicity can be readily discerned from routine experimental variation. The *Escherichia coli* tester strain WP2*uvr*A does not possess the *rfa* wall mutation that the *Salmonella typhimurium* strains have and thus, a different range of cytotoxicity may be observed. Also, the cytotoxicity induced by a test article in the presence of S9 mix may vary greatly from that observed in the absence of S9 mix. Therefore, this would require that different test article dose ranges be tested in the mutagenicity assay based on the presence or absence of the S9 mix.

2. Evaluation of the Dose Rangefinding Study

Cytotoxicity is detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn.

3. Selection of the Maximum Dose for the Mutagenicity Assay

a. No Cytotoxicity Observed

Since no cytotoxicity was observed in the dose rangefinding study, the highest dose of test article used in the mutagenicity assay was the same as that tested in the rangefinding study.

B. Mutagenicity Assay

1. Design

The assay was performed using tester strains TA98, TA100, TA1535, TA1537 and WP2*uvr*A both in the presence and absence of S9 mix. Five doses of test article were tested along with the appropriate vehicle and positive controls. The doses of test article were selected based on the results of the dose rangefinding study.

2. Frequency and Route of Administration

The tester strains were exposed to the test article via the plate incorporation methodology originally described by Ames *et al* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the plate incorporation methodology, the test article, the tester strain and the S9 mix (where appropriate) were combined in molten agar which was overlaid onto a minimal agar plate. Following incubation at 37 ± 2°C for 48 ± 8 hr, revertant colonies were counted. All doses of the test article, the vehicle controls and the positive controls were plated in triplicate.

C. Plating Procedures

These procedures were used in both the dose rangefinding study and the mutagenicity assay.

Each plate was labeled with a code which identified the test article, test phase, tester strain, activation condition and dose. The S9 mix and dilutions of the test article were prepared immediately prior to their use.

When S9 mix was not required, 100 µl of tester strain and 50 µl of vehicle or test article dose was added to 2.5 ml of molten selective top agar (maintained at 45 ± 2°C). When S9 mix was required, 500 µl of S9 mix, 100 µl of tester strain and 50 µl of vehicle or test article dose was added to 2.0 ml of molten selective top agar. After the required components had been added, the

mixture was vortexed and overlaid onto the surface of 25 ml of minimal bottom agar contained in a 15 x 100 mm petri dish. After the overlay had solidified, the plates were inverted and incubated for 48 ± 8 hr at 37 ± 2°C. Positive control articles were plated using a 50 µl plating aliquot.

D. Scoring the Plates

Plates which were not evaluated immediately following the incubation period were held at 5 ± 3°C until such time that colony counting and bacterial background lawn evaluation could take place.

1. Bacterial Background Lawn Evaluation

The condition of the bacterial background lawn was evaluated for evidence of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that dose on the data tables using the code system presented at the end of the Materials and Methods Section.

2. Counting Revertant Colonies

The number of revertant colonies per plate for the vehicle controls and all plates containing test article were counted manually. The number of revertant colonies per plate for the positive controls were counted by automated colony counter.

E. Analysis of Data

For all replicate platings, the mean revertants per plate and the standard deviation were calculated. The results of these calculations are presented in tabular form in the Data Tables Section of this report.

EVALUATION OF TEST RESULTS

Before assay data were evaluated, the criteria for a valid assay had to be met.

A. Criteria For A Valid Assay

The following criteria were used to determine a valid assay:

1. Tester Strain Integrity : *Salmonella typhimurium*

a. *rfa* Wall Mutation

To demonstrate the presence of the *rfa* wall mutation, tester strain cultures exhibited sensitivity to crystal violet.

b. pKM101 Plasmid

To demonstrate the presence of the R-factor plasmid, pKM101, cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin.

c. Characteristic Number of Spontaneous Revertants

To demonstrate the requirement for histidine, the tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. The acceptable ranges for the vehicle controls were as follows:

TA98	8 - 60
TA100	60 - 240
TA1535	4 - 45
TA1537	2 - 25

2. Tester Strain Integrity : *Escherichia coli*

a. Characteristic Number of Spontaneous Revertants

To demonstrate the requirement for tryptophan, the tester strain culture exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. The acceptable range for the WP2*uvr*A vehicle controls was 5 to 40 revertants per plate.

3. Tester Strain Culture Density

To demonstrate that appropriate numbers of bacteria are plated, the density of tester strain cultures were greater than or equal to 0.5×10^9 bacteria per ml and/or had reached a target level of turbidity demonstrated to produce cultures with a density greater than or equal to 0.5×10^9 bacteria per ml.

4. Positive Control Values

a. Positive Control Values in the Absence of S9 Mix

To demonstrate that the tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

b. Positive Control Values in the Presence of S9 Mix (S9 Mix Integrity)

To demonstrate that the S9 mix was capable of metabolizing a promutagen to its mutagenic form(s), the mean value of the positive control for a respective tester strain in the presence of the S9 mix exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

An acceptable positive control in the presence of S9 for a specific strain was evaluated as having demonstrated both the integrity of the S9 mix and the ability of the tester strain to detect a mutagen.

5. Cytotoxicity

A minimum of three non-toxic doses were required to evaluate assay data.

B. Criteria For A Positive Response

Once the criteria for a valid assay had been met, responses observed in the assay were evaluated as follows:

1. Tester Strains TA98 ,TA100, and WP2*uvr*A

For a test article to be considered positive, it had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

2. Tester Strains TA1535 and TA1537

For a test article to be considered positive, it had to produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

RECORDS TO BE MAINTAINED

All raw data, documentation, records, the protocol, and the final report generated as a result of this study will be archived in the storage facilities of Corning Hazleton Inc. for at least one year following submission of the final report to the Sponsor. After the one year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc. for an additional period of time or sent to a storage facility designated by the Sponsor.

REFERENCES

Ames, B.N., J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test. Mutation Research 31:347-364 (1975).

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BACTERIAL BACKGROUND LAWN EVALUATION CODE

The condition of the background bacterial lawn is evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate as follows:

CODE	DEFINITION	CHARACTERISTICS OF BACKGROUND LAWN
1	Normal	A healthy microcolony lawn.
2	Slightly Reduced	A noticeable thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	A marked thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	An extreme thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
5	Absent	A complete lack of any microcolony lawn.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic and/or macroscopic test article precipitate.

Evidence of macroscopic test article precipitate on the plates is recorded by addition of the following precipitate code to the code number used to evaluate the condition of the background bacterial lawn.

sp	Slight Precipitate	Noticeable macroscopic precipitate on the plate, however, the precipitate does not influence automated counting of the plate.
mp	Moderate Precipitate	The amount of macroscopic precipitate on the plate would interfere with automated counting, thus requiring the plate to be hand counted.
hp	Heavy Precipitate	The large amount of macroscopic precipitate on the plate makes the required hand counting difficult.

Example: 4mp would indicate a plate observed to have an extremely reduced background lawn which had to be counted manually due to the marked amount of macroscopic test article precipitate.

SECTION IV. RESULTS AND CONCLUSIONS

RESULTS

A. Test Article Handling

The test article, **T-6357**, was stored at room temperature. Deionized water (CHV lots 336 and 337) was used as the vehicle. At 100 mg per ml, which was the most concentrated stock dilution prepared, the test article formed a clear colorless solution. The test article remained a solution in all succeeding dilutions prepared for the mutagenicity assay.

B. Dose Rangefinding Study

Doses to be tested in the mutagenicity assay were selected based on the results of the dose rangefinding study conducted on the test article using tester strains TA100 and WP2*uvr*A in both the presence and absence of S9 mix (one plate per dose). Ten doses of test article, from 5,000 to 6.67 µg per plate, were tested and the results are presented in Tables 1 and 2. These data were generated in Experiment 17387-A1. No cytotoxicity was observed in either the presence or absence of S9 mix as evidenced by a normal background lawn and no decrease in the number of revertants per plate.

C. Mutagenicity Assay

The mutagenicity assay results for **T-6357** are presented in Tables 3 and 4. These data were generated in Experiment 17387-B1. The data are presented as mean revertants per plate ± standard deviation for each treatment and control group (Table 4) and as individual plate counts (Table 3).

The results of the dose rangefinding study were used to select five doses to be tested in the mutagenicity assay. The doses tested were 5,000, 3,330, 1,000, 333, and 100 µg per plate in both the presence and absence of S9 mix.

In Experiment 17387-B1 (Tables 3 and 4), all data were acceptable and no positive increases in the number of revertants per plate were observed with any of the tester strains either in the presence or absence of S9 mix.

All criteria for a valid study were met.

CONCLUSIONS

The results of the *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay indicate that, under the conditions of this study, 3M's test article, **T-6357**, did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9).

SECTION V. DATA TABLES

TABLE 1

DOSE RANGEFINDING STUDY

TEST ARTICLE ID: T-6357

EXPERIMENT ID: 17387-A1 DATE PLATED: 04-Feb-96

VEHICLE: Deionized water DATE COUNTED: 07-Feb-96

	TA100 REVERTANTS PER PLATE			
	WITH S9		WITHOUT S9	
µg/PLATE	REVERTANTS PER PLATE	BACKGROUND LAWN EVALUATION*	REVERTANTS PER PLATE	BACKGROUND LAWN EVALUATION*
0.00 (Vehicle) (50 µl)	97	1	102	1
Test Article				
6.67	125	1	85	1
10.0	87	1	115	1
33.3	112	1	87	1
66.7	108	1	88	1
100	103	1	98	1
333	82	1	102	1
667	125	1	86	1
1000	136	1	93	1
3330	114	1	120	1
5000	135	1	105	1

* Background Lawn Evaluation Codes:

1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

TABLE 2

DOSE RANGEFINDING STUDY

TEST ARTICLE ID: T-6357

EXPERIMENT ID: 17387-A1 DATE PLATED: 04-Feb-96

VEHICLE: Deionized water DATE COUNTED: 07-Feb-96

µg/PLATE	WP2uvrA REVERTANTS PER PLATE WITH S9		WITHOUT S9	
	REVERTANTS PER PLATE	BACKGROUND LAWN EVALUATION*	REVERTANTS PER PLATE	BACKGROUND LAWN EVALUATION*
0.00 (Vehicle) (50 µl)	14	1	11	1
Test Article				
6.67	13	1	22	1
10.0	26	1	19	1
33.3	24	1	13	1
66.7	21	1	13	1
100	16	1	20	1
333	20	1	22	1
667	11	1	13	1
1000	21	1	11	1
3330	19	1	18	1
5000	16	1	13	1

* Background Lawn Evaluation Codes:

1 = normal; 2 = slightly reduced; 3 = moderately reduced

4 = extremely reduced; 5 = absent; 6 = obscured by precipitate

sp = slight precipitate; mp = moderate precipitate (requires hand count); hp = heavy precipitate (requires hand count)

TABLE 3
MUTAGENICITY ASSAY RESULTS
INDIVIDUAL PLATE COUNTS

TEST ARTICLE ID: T-6357

EXPERIMENT ID: 17387-B1

DATE PLATED: 14-Feb-96 VEHICLE: Deionized water

DATE COUNTED: 21-Feb-96 PLATING ALIQUOT: 50 µl

		REVERTANTS PER PLATE															BACKGROUND LAWN*
DOSE/PLATE		TA98			TA100			TA1535			TA1537			WP2uvrA			
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
MICROSOMES: Rat Liver																	
VEHICLE CONTROL		29	36	23	130	140	131	10	9	9	4	7	8	11	17	6	1
TEST ARTICLE	100 µg	30	32	24	135	143	149	12	14	12	10	9	8	15	7	14	1
	333 µg	26	37	16	113	99	127	12	16	14	3	10	9	13	13	14	1
	1000 µg	15	16	17	122	119	138	11	23	11	10	3	7	14	11	13	1
	3330 µg	26	27	18	134	146	132	12	16	14	5	10	2	10	20	19	1
	5000 µg	19	24	24	139	129	117	10	11	14	4	4	9	16	15	21	1
POSITIVE CONTROL **		1086	1106	1231	1400	1443	1450	227	206	348	134	101	130	393	344	372	1
MICROSOMES: None																	
VEHICLE CONTROL		20	17	8	84	114	79	5	12	11	6	4	8	13	14	12	1
TEST ARTICLE	100 µg	11	12	9	93	106	84	14	12	9	2	4	5	20	10	14	1
	333 µg	15	12	11	91	90	80	8	8	10	7	9	5	12	6	12	1
	1000 µg	14	18	12	101	77	98	7	11	13	3	11	6	14	11	9	1
	3330 µg	6	17	9	77	85	98	13	16	10	3	5	4	14	12	13	1
	5000 µg	9	20	11	84	93	86	13	15	10	4	7	5	17	13	7	1
POSITIVE CONTROL ***		158	151	162	726	734	779	646	599	701	463	553	742	124	153	119	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2uvrA 2-aminoanthracene 25.0 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced
4 = extremely reduced 5 = absent 6 = obscured by precipitate
sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

TABLE 4
MUTAGENICITY ASSAY RESULTS
SUMMARY

TEST ARTICLE ID: T-6357

EXPERIMENT ID: 17387-B1

DATE PLATED: 14-Feb-96 VEHICLE: Deionized water

DATE COUNTED: 21-Feb-96 PLATING ALIQUOT: 50 µl

		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
	DOSE/PLATE	TA98		TA100		TA1535		TA1537		WP2uvrA		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL		29	7	134	6	9	1	6	2	11	6	1
TEST ARTICLE	100 µg	29	4	142	7	13	1	9	1	12	4	1
	333 µg	26	11	113	14	14	2	7	4	13	1	1
	1000 µg	16	1	126	10	15	7	7	4	13	2	1
	3330 µg	24	5	137	8	14	2	6	4	16	6	1
	5000 µg	22	3	128	11	12	2	6	3	17	3	1
POSITIVE CONTROL **		1141	79	1431	27	260	77	122	18	370	25	1
MICROSOMES: None												
VEHICLE CONTROL		15	6	92	19	9	4	6	2	13	1	1
TEST ARTICLE	100 µg	11	2	94	11	12	3	4	2	15	5	1
	333 µg	13	2	87	6	9	1	7	2	10	3	1
	1000 µg	15	3	92	13	10	3	7	4	11	3	1
	3330 µg	11	6	87	11	13	3	4	1	13	1	1
	5000 µg	13	6	88	5	13	3	5	2	12	5	1
POSITIVE CONTROL ***		157	6	746	29	649	51	586	142	132	18	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
WP2uvrA 2-aminoanthracene 25.0 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

CORNING Hazleton

MUTAGENICITY TEST ON

T-6357

IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY

FINAL REPORT

AUTHOR

Hemalatha Murli, Ph.D.

PERFORMING LABORATORY

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LABORATORY PROJECT IDENTIFICATION

CHV Study No.: 17387-0-455

SUBMITTED TO

3M
3M Center, Building 220-2E-02
St. Paul, Minnesota 55144-1000

STUDY COMPLETION DATE

April 23, 1996

QUALITY ASSURANCE STATEMENT

Project Title: *In Vivo* Mouse Micronucleus Assay

Project No.: 20996 Assay No.: 17387

Protocol No.: 455 Edition No.: 17

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the Standard Operating Procedures of the Quality Assurance Unit and according to the general requirements of the appropriate Good Laboratory Practice regulations. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspection/Date	Findings Reported	Auditor
Harvest/02/28/1996	02/28/1996	C. Orantes
Draft Report Review/04/18,19/1996	04/19/1996	C. Orantes
Final Report Review/04/23/1996	04/23/1996	C. Orantes

Quality Assurance Unit 4/23/96
Date Released

STUDY COMPLIANCE AND CERTIFICATION

The described study was conducted in compliance with the Good Laboratory Practice regulations as set forth in the Food and Drug Administration (FDA) Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978, (effective June 20, 1979) with any applicable amendments. There were no significant deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria.

All test and control results in this report are supported by an experimental data record and this record has been reviewed by the Study Director. All raw data, documentation, records, protocol and a copy of the final report generated as a result of this study will be archived in the storage facilities of Corning Hazleton Inc. for at least one year following submission of the final report to the Sponsor. After the one year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc. for an additional period of time, or sent to a storage facility designated by the Sponsor.

Submitted By:

Study Director:

HMurli

Hemalatha Murli, Ph.D.
Mammalian Cytogenetics
Department of Genetic and Cellular Toxicology

4/23/96

Study Completion Date

TABLE OF CONTENTS

SUMMARY

Mutagenicity Test on T-6357 in an *In Vivo* Mouse Micronucleus Assay

The objective of this *in vivo* assay was to evaluate the ability of the test article, T-6357, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice.

In the dose selection study, the test article was solubilized in deionized water and dosed by oral gavage at 200, 400, 610, 810, and 1010 mg/kg in Trial I and no toxic signs were observed in any of the animals. In Trial II, animals were dosed by oral gavage at 1500, 2375, 3250, 4125 and 5000 mg/kg. Six animals (three males and three females) were assigned to each dose group. Animals were observed for three days after dosing for toxic signs and/or mortality.

Based on the results of the dose selection study, the maximum tolerated dose was estimated as 2000 mg/kg. In the micronucleus assay, the test article was solubilized in deionized water and dosed oral gavage at 500, 1000, and 2000 mg/kg. Ten animals (five males and five females) were randomly assigned to each dose/harvest time group. Vehicle and positive control groups euthanatized approximately 24 hours after dosing were included in the assay. The animals dosed with the test article were euthanatized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow.

The test material, T-6357, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse bone marrow micronucleus test.

Mutagenicity Test on T-6357 in an *in vivo* Mouse Micronucleus Assay

1.0 SPONSOR: 3M

2.0 MATERIAL (Test Article)

2.1 Client's Identification: T-6357

2.2 Date Received: January 16, 1996

2.3 Physical Description: Clear, amber liquid

2.4 Genetics Assay No.: 17387

3.0 TYPE OF ASSAY: *In Vivo* Mouse Micronucleus Assay

4.0 PROTOCOL NO.: 455, Edition 17

5.0 STUDY DATES

5.1 Initiation Date: January 18, 1996

5.2 Experimental Start Date: February 14, 1996

5.3 Experimental Termination Date: March 22, 1996

6.0 SUPERVISORY PERSONNEL

6.1 Study Director: Hemalatha Murli, Ph.D.

6.2 Laboratory Supervisor: Monica Vegarra, B.S.

7.0 OBJECTIVE

The objective of this *in vivo* assay was to evaluate the ability of the test article, T-6357, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice. This study was conducted using modifications of the procedures suggested by Heddle et al. (1983).

8.0 MATERIALS

Adult male and female mice, strain Crl:CD-1®(ICR) BR, were purchased from Charles River Laboratories, Portage MI. This healthy, random bred strain was selected to maximize genetic heterogeneity and at the same time assure access to a common source. The protocol for this study was approved by the CHV-ACUC prior to the initiation of dosing.

Animals were housed seven per cage during quarantine, and housed five at randomization. The temperature and relative humidity were maintained at 72±6°F and 55±15%, respectively, except on February 19, 1996, for the dose selection studies, when the relative humidity was recorded at 31.1% and on the following dates for the micronucleus assay: February 17, 18, 19, 24, 24, and 25, 1996 when the relative humidity was recorded at 39.1%, 37.3%, 34.5%, 32.5, 34.4%, and 20.8%, respectively. A 12-hour light/12-hour dark cycle was maintained. A commercial diet (Purina® Certified Laboratory Pellets ® # 5002) and water were available ad libitum for the duration of the study. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water was analyzed on a retrospective basis for specified microorganisms, pesticides, alkalinity, heavy metals, and halogens. Sanitized caging was used for housing the animals. Personnel handling animals or working within the animal facilities were required to wear suitable protective garments and equipment.

Animals were quarantined for at least seven days before being placed on study. Animals were randomly assigned to study groups and were individually weighed prior to dosing. All animals were dosed based upon the individual body weights. Animals were uniquely identified by ear tag. Dose or treatment groups were identified by cage card/label.

At the termination of the study all surviving animals were euthanatized by CO_2 followed by penetration of the thorax. Any extra animals not used for the study were used for training purposes.

9.0 SOLUBILITY AND STABILITY:

The test article, T-6357, was supplied as a clear amber liquid. The solubility of the test article was evaluated in deionized water. A clear, light yellow solution was obtained at a concentration of about 391.6 mg/ml. Deionized water was the vehicle of choice for this assay. The stability of the test material under the dosing conditions of this assay is the responsibility of the sponsor.

10.0 DOSE SELECTION STUDY

TRIAL I

10.1 Dose Selection

Dose levels of 200, 400, 610,810 and 1010 mg/kg were administered by oral gavage for the dose selection study.

10.2 Dosing Information

The animals used in the dose selection assay were dosed on February 14, 1996. The weight range of the animals used in the dose range finding assay was 28.5-36.6 and 22.9-27.6 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 100 mg/ml stock for the high dose (1000 mg/kg). This was prepared by adding 10.98ml of deionized water (Lot # 19, prepared at CHV) to 1.2081 g of T-6357, resulting in a clear pale yellow solution with a final volume of 12.0 ml. Dilutions of this stock were prepared for the remaining dose levels.

Dosing was achieved using a 10.0 ml/kg dosing volume. All animals were nine weeks old at the time of dosing. An outline of the dosing scheme is found in the following table.

A total of 30 animals was used in this assay.

	DOSE GROUPS	
TREATMENT	M	F
T-6357		
200 mg/kg	3	3
400 mg/kg	3	3
610 mg/kg	3	3
810 mg/kg	3	3
1010 mg/kg	3	3

All doses given were on an acute (one-time only) basis.

10.3 Results and Interpretation

All animals were examined after dosing and daily throughout the duration of the study (three days) for toxic effects and/or mortalities. Immediately following dosing all animals appeared normal and remained healthy until the end of the observation period.

10.4 Conclusion

Based on these results, the maximum tolerated dose could not be determined.

TRIAL II

10.5 Dose Selection

Dose levels of 1500, 2375, 3250, 4125 and 5000 mg/kg were administered by oral gavage for the dose selection study.

10.6 Dosing Information

The animals used in the dose selection assay were dosed on February 15, 1996. The weight range of the animals used in the dose range finding assay was 28.5-36.4 and 23.6-27.5 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 500 mg/ml stock for the high dose (5000 mg/kg). This was prepared by adding deionized water (Lot # 19, prepared at CHV) to 7.4993 g of T-6357 up to a volume of 15.0 ml, resulting in a clear pale yellow solution. Dilutions of this stock were prepared for the 1500, 2375, 3250, and 4125 mg/kg dose levels.

Dosing was achieved using a 10.0 ml/kg dosing volume. All animals were nine weeks and one day old at the time of dosing. An outline of the dosing scheme is found in the following table.

A total of 30 animals was used in this assay.

TREATMENT	DOSE GROUPS M	F
T-6357		
1500 mg/kg	3	3
2375 mg/kg	3	3
3250 mg/kg	3	3
4125 mg/kg	3	3
5000 mg/kg	3	3

All doses given were on an acute (one-time only) basis.

10.7 Results and Interpretation

All animals were examined after dosing and daily throughout the duration of the study (three days) for toxic effects and/or mortalities.

Immediately after dosing, in the 1500 mg/kg dose group, 4 animals were hypoactive, and the other 2 were normal. In the 2375 mg/kg dose group, 2 males were languid and ataxic, and the remaining animals were normal and healthy. In the 3250 mg/kg dose group, 2 males and 1 female were languid and ataxic, and the remaining animals were normal and healthy. In the 4125 mg/kg dose group, 1 male was prostrate with labored breathing, and the remaining animals were hypoactive. In the 5000 mg/kg dose group, 1 male and 1 female were prostrate with labored breathing, and the remaining animals were slightly hypoactive.

Approximately 1 hour after dosing, all animals from the 1500 mg/kg dose group were normal and healthy. In the 2375 mg/kg dose group, 1 male and 1 female were hypoactive, and the remaining animals were normal and healthy. In the 3250 mg/kg dose group, 1 male and 2 females were hypoactive and the remaining animals were normal and healthy. All animals from the 4125 mg/kg dose group were hypoactive. In the 5000 mg/kg dose group, 1 male and 2 females were prostrate, and the remaining animals were hypoactive.

Approximately 17 hours after dosing, all animals from the 1500 mg/kg dose group were normal and healthy. All animals from the 2375 mg/kg were hypoactive and hunched, and 2 males (#'s 6336, 6334) also had squinted eyes with blood colored staining on eyes. All 3250 mg/kg dose level animals were hypoactive and

hunched, and 2 males (#'s 6333, 6337) also had blood colored staining on eyelids. All 4125 mg/kg animals were hypoactive with dyspnea and blood colored staining on eyelids. All 5000 mg/kg animals were hypoactive with dyspnea; all males also had tremors, chromodacryorrhea, and were cold to the touch. Their cage also had several blood colored spots in it. One female (# 6340) had urine colored stains, profound foot splay, and was cold to the touch. A second female also had a blood colored glaze on the eyelids and was cold to the touch.

Approximately 42.5 hours after dosing, all animals from the 1500 mg/kg dose group and all females from the 2375 mg/kg and 3250 mg/kg dose groups were normal and healthy. One 2375 mg/kg dose group male (# 6336) was found dead and the remaining males were hypoactive. One 3250 mg/kg dose group male (# 6337) was found dead and the remaining males were hypoactive. In the 4125 mg/kg dose group, 2 males (# 6332, 6338) and one female (# 6355) were found dead; the remaining male were hypoactive, hunched with dyspnea; the remaining female was hypoactive. In the 5000 mg/kg dose group, 1 male (# 6340) and 1 female (# 6360) were found dead; the remaining males were hypoactive; the remaining females were hypoactive and had tremors, squinted eyes and dyspnea.

Approximately 66.5 hours after dosing, all animals from the 1500 mg/kg, and all surviving animals from the 2375 mg/kg and 3250 mg/kg dose groups were normal and healthy. All surviving animals in the 4125 mg/kg dose group were hypoactive with dyspnea. In the 5000 mg/kg dose group, 2 females (#'s 6346, 6352) were found dead and the surviving males were hypoactive, hunched and had dyspnea.

Approximately 71.5 hours after dosing, 1 male from the 5000 mg/kg dose group (# 6342) was found dead.

Approximately 91 hours after dosing, 1 male (# 6344) from the 2375 mg/kg dose group, 1 male from the 3250 mg/kg dose group and 1 male (# 6339) from the 4125 mg/kg dose group were found dead. All remaining animals from the 1500, 2375 and 3250 mg/kg and 4125 mg/kg female dose groups were normal and healthy. The surviving male (# 6343) from the 5000 mg/kg dose group appeared pale, hunched and hypoactive.

The mortality data for this assay are summarized in the following table:

Summary of Mortalities Within 3 Days in Mice Dosed Acutely with T-6357

Observations

Treatment	Male	Female
1500 mg/kg	0/3	0/3
2375 mg/kg	2/3	0/3
3250 mg/kg	2/3	0/3
4125 mg/kg	3/3	1/3
5000 mg/kg	2/3	3/3

10.8 Conclusion

Based on these results, the maximum tolerated dose was estimated to be 2000 mg/kg.

11.0 MICRONUCLEUS STUDY

11.1 Dose Selection

Based on results from the dose selection study, dose levels of 500, 1,000, and 2,000 mg/kg were selected for testing in this study.

11.2 Micronucleus Assay Dosing Information

The animals used in the micronucleus assay were dosed on February 27, 1996. Cyclophosphamide (CAS # 6055-19-2; Sigma, Lot # 44H0486), the positive control, was solubilized in deionized water (Lot # 19, prepared at CHV) and was administered by oral gavage at 80.0 mg/kg. The vehicle control, deionized water (Lot # 19, prepared at CHV), was administered concurrently with the test article at a volume of 10.0 ml/kg. The weight range of the animals used in the micronucleus assay was 29.2-39.5 and 23.0-32.2 grams for the males and females, respectively. The dosing solutions for the assay were prepared by making a 200 mg/ml stock for the high dose (2000 mg/kg). This was prepared by adding deionized water to 5.0088 g of T-6357 up to a volume of 25 ml. A clear pale

yellow solution was obtained at a concentration of 200 mg/ml. Dilutions of this stock were prepared for the remaining dose levels. A second group of animals (designated Secondary Dose Group) was also assigned to the study and was dosed at the high dose selected. These animals were only used in the assay as replacements for any which died in the primary dose group.

Ten animals (five males and five females) were randomly assigned to each dose/-harvest time group. Vehicle and positive control groups, euthanatized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanatized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow. An outline of the dosing scheme is found in the following table:

Dosing Scheme for Micronucleus Assay

A total of 120 animals was used in this assay

	Number of Animals Assigned							
	Primary Dose Groups						Secondary Dose Groups[a]	
	24 Hr		48 Hr		72 Hr			
Treatment	M	F	M	F	M	F	Male	Female
T-6357								
500 mg/kg	5	5	5	5	5	5	-	-
1000 mg/kg	5	5	5	5	5	5	-	-
2000 mg/kg	5	5	5	5	5	5	5	5
Vehicle Control, deionized water, 10.0 ml/kg	5	5	-	-	-	-	-	-
Positive Control, Cyclophosphamide, 80.0 mg/kg	5	5	-	-	-	-	-	-

[a] The animals assigned to the secondary dose groups were dosed and were only used to replace animals which died in the primary dose group at the high dose level. All extra animals not used as replacements were euthanatized at the completion of the trial.

The age of the animals at the time of dosing was nine weeks and one day.

Volumes dosed were 10.0 ml/kg and were based upon individual animal weights.

12.0 BONE MARROW HARVEST, SLIDE PREPARATION AND ANALYSIS

At the appropriate harvest time, the animals were euthanatized with CO_2 followed by penetration of the thorax and the adhering soft tissue and epiphyses of both femora were removed. The marrow was flushed from the bone and transferred to centrifuge tubes containing 3 - 5 ml bovine serum (one tube for each animal). Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air dried. The slides were fixed in methanol, and stained in May-Grunwald solution followed by Giemsa (Schmid, 1975). The air-dried slides were coverslipped using Depex® mounting medium.

The slides were coded for analysis, and scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. Standard forms were used to record these data. One thousand PCEs per animal were scored. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The normal frequency of micronuclei in this Crl:CD-1®(ICR) BR strain is about 0.0-0.4%.

The frequency of PCEs versus NCEs was determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring the first 1000 erythrocytes.

13.0 EVALUATION CRITERIA:

13.1 General

The criteria for the identification of micronuclei were those of Schmid (1976). Micronuclei were darkly stained and generally round, although almond and ringshaped micronuclei occasionally occurred. Micronuclei had sharp borders and were generally between 1/20 and 1/5 the size of the PCE. The unit of scoring was the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-grey and red, respectively).

13.2 Data Presentation and Interpretation

Data are summarized by sex and dose groups for the different time points. Individual animal data are also presented. The analysis of these data was performed using an analysis of variance (Winer, 1971) on either untransformed (when variances are homogeneous) and rank transformed (when variances are

heterogeneous) proportions of cells with micronuclei per animal. If the analysis of variance was significant ($p<0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were significantly different from the negative control. Analyses were performed separately for each harvest time and sex combination. The criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level was considered negative. In either case, the final decision was based on scientific judgment.

14.0 RESULTS AND INTERPRETATION:

All animals were observed immediately after dosing and periodically throughout the duration of the assay for toxic symptoms and/or mortalities. All animals in the vehicle and positive control groups appeared normal after dosing and remained healthy until the appropriate harvest times. All test article dosed groups appeared normal immediately after dosing and remained healthy until the appropriate harvest times, except for one 72 hour male at the 1000 mg/kg dose level (# 6562), which was found dead about 72 hours post dosing.

The test article, T-6357, induced no significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls in either sex or at any of the harvest times. Due to toxicity, a significant reduction was observed in the PCE/NCE ratios of the males from the 500, 1000, and 2000 mg/kg dose groups at the 72 hour harvest time. The positive control, CP, induced significant increases in micronucleated PCEs in both sexes as compared to the vehicle controls, with means and standard errors of 7.98% ± 1.30% and 4.16% ± 0.92% for the males and females, respectively. The data summarized by dose group are presented in Table 1 and individual animal data are found in Tables 2 through 7. Historical control data are presented in Table 8.

15.0 CONCLUSION:

The test material, T-6357, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse micronucleus assay.

16.0 REFERENCES:

Dunnett, C.W.: A multiple comparisons procedure for comparing several treatments with a control. J. Am. Statist. Assoc., 50:1096-1121, 1955.

Dunnett, C.W.: New tables for multiple comparisons with a control. Biometrics, 20:482-491, 1964.

Heddle, J.A., Hite, M., Kirkhart, B., Larsen, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F.: The induction of micronuclei as a measure of genotoxicity. Mutation Res., 123:61-118, 1983.

Schmid, W.: The micronucleus test. Mutation Res., 31:9-15, 1975.

Schmid, W.: The micronucleus test for cytogenetic analysis. Chemical Mutagens: Principles and Methods for Their Detection, Vol. 4 (A. Hollaender, ed.). Plenum, pp. 31-53, 1976.

Winer, B.J.: Statistical Principles in Experimental Design, McGraw-Hill, New York, Second Edition, 1971.

17.0 DEVIATIONS FROM THE SIGNED PROTOCOL

The following deviations were made from the signed protocol.

1. On February 19, 1996, for the dose selection studies, the relative humidity was recorded at 31.1%. For the micronucleus assay: on February 17, 18, 19, 24, 24, and 25, 1996, the relative humidity was recorded at 39.1%, 37.3%, 34.5%, 32.5%, 34.4%, and 20.8%, respectively. This did not affect the animals and there was no impact on the integrity of this study.

2. Due to a technical error in the preparation of dosing stocks for Trial I of the dose selection study, the actual dose levels used were 610, 810, and 1010 mg/ml. The difference is <2% and had no impact on the integrity of this study.

18.0 EXPERIMENT DATA TABLES

TABLE 1
MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY: 17387

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
CONTROLS							
VEHICLE	Water	24 hr	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.67 ± 0.05	0.71 ± 0.08
POSITIVE	CP 80.0 mg/kg	24 hr	7.98 ± 1.30*	4.16 ± 0.92*	6.07 ± 0.98*	0.78 ± 0.08	0.71 ± 0.05
TEST ARTICLE	500 mg/kg	24 hr	0.02 ± 0.02	0.10 ± 0.03	0.06 ± 0.02	0.72 ± 0.09	0.73 ± 0.02
		48 hr	0.04 ± 0.04	0.02 ± 0.02	0.03 ± 0.02	0.54 ± 0.12	0.62 ± 0.03
		72 hr	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.43 ± 0.06**	0.52 ± 0.03
	1000 mg/kg	24 hr	0.10 ± 0.03	0.04 ± 0.02	0.07 ± 0.02	0.63 ± 0.05	0.77 ± 0.04
		48 hr	0.10 ± 0.05	0.04 ± 0.02	0.07 ± 0.03	0.65 ± 0.06	0.56 ± 0.03
		72 hr	0.03 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.29 ± 0.04**	0.58 ± 0.06
	2000 mg/kg	24 hr	0.06 ± 0.06	0.10 ± 0.04	0.08 ± 0.04	0.61 ± 0.07	0.70 ± 0.07
		48 hr	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.67 ± 0.04	0.69 ± 0.04
		72 hr	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.36 ± 0.03**	0.53 ± 0.04

* Significantly greater than the corresponding vehicle control, $p<0.05$.
** Significantly lower than the corresponding vehicle control, $p<0.05$.

CP = Cyclophosphamide

TABLE 2
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
24 HOUR HARVEST	MALE				
VEHICLE CONTROL	Water		6556	0	0.47
			6560	0	0.65
			6589	1	0.72
			6595	0	0.71
			6605	0	0.78
POSITIVE CONTROL	CP 80.0	mg/kg	6548	125	0.58
			6553	63	1.06
			6573	90	0.63
			6579	71	0.78
			6580	50	0.83
TEST ARTICLE	500	mg/kg	6551	0	1.01
			6581	0	0.54
			6585	1	0.86
			6597	0	0.57
			6598	0	0.65
	1000	mg/kg	6550	0	0.72
			6561	1	0.66
			6569	1	0.56
			6572	1	0.72
			6574	2	0.48
	2000	mg/kg	6547	0	0.78
			6549	3	0.63
			6587	0	0.55
			6599	0	0.40
			6602	0	0.70

CP = Cyclophosphamide
MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 3
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
24 HOUR HARVEST	**FEMALE**				
VEHICLE CONTROL	Water		6612	0	0.57
			6617	0	0.96
			6625	1	0.75
			6637	1	0.75
			6647	0	0.52
POSITIVE CONTROL	CP 80.0	mg/kg	6616	27	0.81
			6633	29	0.75
			6641	24	0.78
			6656	64	0.69
			6658	64	0.53
TEST ARTICLE	500	mg/kg	6607	2	0.73
			6610	1	0.79
			6626	1	0.60
			6642	1	0.72
			6646	0	0.79
	1000	mg/kg	6611	1	0.76
			6615	0	0.92
			6634	1	0.65
			6649	0	0.78
			6666	0	0.75
	2000	mg/kg	6621	1	0.54
			6627	0	0.76
			6644	0	0.88
			6653	2	0.56
			6665	2	0.77

CP = Cyclophosphamide
MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 4
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
48 HOUR HARVEST	MALE				
TEST ARTICLE	500	mg/kg	6554	0	0.77
			6557	0	0.51
			6568	0	0.72
			6583	0	0.60
			6594	2	0.12
	1000	mg/kg	6566	0	0.58
			6570	1	0.49
			6582	3	0.77
			6601	1	0.81
			6603	0	0.61
	2000	mg/kg	6552	1	0.78
			6584	0	0.64
			6591	0	0.66
			6596	0	0.56
			6604	0	0.71

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 5
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
48 HOUR HARVEST	FEMALE				
TEST ARTICLE	500	mg/kg	6613	0	0.60
			6628	1	0.72
			6648	0	0.56
			6651	0	0.61
			6661	0	0.61
	1000	mg/kg	6638	0	0.55
			6639	1	0.47
			6640	0	0.53
			6645	0	0.59
			6652	1	0.64
	2000	mg/kg	6608	1	0.78
			6620	0	0.68
			6624	1	0.78
			6629	0	0.59
			6664	0	0.63

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 6
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
72 HOUR HARVEST	MALE				
TEST ARTICLE	500	mg/kg	6563	0	0.35
			6571	0	0.30
			6577	0	0.51
			6586	0	0.39
			6606	0	0.61
	1000	mg/kg	6558	1	0.27
			6559	0	0.19
			6562*		
			6565	0	0.31
			6588	0	0.40
	2000	mg/kg	6564	0	0.39
			6567	0	0.37
			6576	1	0.46
			6578	0	0.35
			6592	0	0.24

* Animal found dead

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 7
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
72 HOUR HARVEST	**FEMALE**				
TEST ARTICLE	500	mg/kg	6622	0	0.56
			6631	0	0.58
			6636	0	0.53
			6657	0	0.45
			6659	1	0.46
	1000	mg/kg	6609	0	0.76
			6614	0	0.64
			6618	0	0.59
			6619	0	0.51
			6643	0	0.41
	2000	mg/kg	6623	1	0.66
			6635	0	0.41
			6654	0	0.54
			6655	1	0.57
			6662	0	0.45

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 8

MOUSE MICRONUCLEUS HISTORICAL CONTROL DATA 7/95 THROUGH 12/95

		% MICRONUCLEATED PCEs PER 1000 PCE MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:NCE MEAN ± S.E.	
		MALES	FEMALES	TOTAL	MALES	FEMALES
POOLED VEHICLE CONTROLS						
	MIN	0.00	0.00	0.01	0.31	0.24
	MAX	0.22	0.24	0.17	0.85	1.03
	AVG	0.087 ± 0.007	0.081 ± 0.008	0.084 ± 0.005	0.550 ± 0.021	0.587 ± 0.025
	N	47	47	47	47	47
POSITIVE CONTROLS Cyclophosphamide, 80.0 mg/kg						
	MIN	2.00	1.50	2.41	0.41	0.40
	MAX	5.68	6.36	5.38	0.72	0.79
	AVG	3.682 ± 0.240	3.170 ± 0.245	3.426 ± 0.184	0.577 ± 0.020	0.588 ± 0.026
	N	19	19	19	19	19

PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Corning Hazleton Inc.
9200 Leesburg Pike
Vienna, Virginia 22182-1699
703 893.5400
703 759.6947 Fax

April 19, 1996

CORNING Hazleton

Steven C. Gordon, Ph.D., DABT
3M Medical Department
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

RE: DRAFT REPORT AND PROTOCOL AMENDMENTS
In Vivo Micronucleus Assay
Protocol No.: 455CO, Ed. No.: 4, Modified for Sponsor
Genetics Assay No.: 17387, 17387-1
Test Material: T-6357

Dear Dr. Gordon:

Enclosed please find two (2) copies of the above referenced report. The report includes an unsigned Quality Assurance Statement and Compliance and Certification Statement, which will be signed upon issuance of the Final Report.

The final report will be issued after your review and notification to us. Please contact the undersigned with any questions, comments, or necessary revisions at your earliest convenience. The report will be finalized after 1 year if no notification is received.

Thank you for giving us this opportunity to work with you.

Sincerely,

CORNING Hazleton

Hema Murli

Hema Murli, Ph.D.
Mammlian Cytogenetics
Department of Genetic and
Cellular Toxicology

HM/paj
enclosures

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-135

Study Number: AMDT-020795.1
Test Substance: FC-120 (T-6052)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

Samples of liver at 28 days post dermal doses of FC-120 (T-6052) were analyzed by combustion for total organic fluorine. Even the highest dose of 1000 mg/kg (50 ug/kg) resulted in no organic fluorine at practical quantitation levels. There is a trace of some fluorine detectable if one uses just relative meter readings. Electrospray mass spectrometry was able to detect m/z=599 ion which is the perfluorodecanesulfonate anion.

The doses were too low to assess dermal absorption with this test method.

2.0 INTRODUCTION

The pharmacokinetic study for FC-120 was not successful. The highest dose was 50 ug/kg. Thus, in this study, if there was a substantial amount of organic fluorine present at 28 days it would indicate that a significant absorption of FC-120 had occurred. However, in the event of very little organic fluorine at 28 days, it would not be possible to make an assessment of dermal absorption. Liver samples and serum samples were available for analysis of total organic fluorine and perfluorodecanesulfonate anion. The samples were analyzed. Because the doses are quite low (high dose 50 ug/kg), it was not expected that fluorine would be detected.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver, bovine serum, rabbit serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver, bovine serum and rabbit serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company, rabbit serum from AMDT-110394.1 (HWI#6329-123) control group animals.

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20±10° C.

3.6 Disposition Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-135), were available for analysis for fluorine compounds. At the discretion of the study director, a series of analytical tests could be performed. The screening for fluoride in liver via combustion was the most likely analysis to present definitive data for absorption if the pharmacokinetic test (IV administration) was positive for fluorine in the liver. Other available tests were electrospray mass spectroscopy. However, if the definitive results could be obtained with combustion analysis alone, only the liver samples would be analyzed and any other tests would be for confirmation.

5.0 EXPERIMENTAL - METHODS

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

5.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

6.0 DATA ANALYSIS

The data from combustion analysis are attached. There does not appear to be any total organic fluorine above the practical quantitation limit for any of the liver samples at 48 hours post intravenous dose for any of the dosing regimens. Even the 50 ug/kg dose is not above the practical quantitation limit. However, if one uses just the meter readings and compares those reading to the values for the controls, there is possibly a trace of fluorine being detected.

Electrospray mass spectrometry analysis is attached. The electrospray was able to detect perfluorodecanesulfonate anion in the 50 ug/kg dose livers. The amount present was not quantitated.

In view of the failure of the pharmacokinetic study (HWI#6329-134) to show a good marker for FC-120 at a dose of 50 ug/kg, it is not reasonable to make an assessment of the extent of dermal absorption from this study at the same dose level.

Other data was collected using Skalar segmented flow analyzer with ion selective electrode (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances that May Have Affected the Quality of the Data: The problem with this analysis is that the pharmacokinetic study did not provide a good marker for dermal absorption because the dose level was too low. The dermal study is not at a higher dose.

7.0 CONCLUSION

This study does not provide a useful assessment of dermal absorption of FC-120. There is not a useful marker.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-135: "Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-020795.1

9.1.3 Amendment to Analytical Protocol AMDT-020795.1

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-020795.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.4 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.1.1 Protocol and Final Report: HWI#6329-135: "Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 27, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-135

Phone 608 241-4471 Fax 60
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON, W.

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
12/21/94	12/21/94	Protocol Review	12/22/94	01/10/95
01/30/95	01/30/95	Protocol Amendment	01/30/95	02/10/95
02/02/95	02/02/95	Body Weight	02/02/95	03/10/95
03/29/95	03/29/95	Data/Report Review	03/29/95	04/10/95
03/29/95	03/29/95	Data Review	03/29/95	04/10/95
06/27/95	06/27/95	Report Rereview	06/27/95	07/10/95

Randy Lenty
Representative, Quality Assurance Unit

6.27.95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

Test Material	T-6052
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 30, 1994
Experimental (In-life) Start Date	January 5, 1995
In-life End Date	February 2, 1995
Experimental Termination Date	June 27, 1995
Study Completion Date	June 27, 1995

KEY PERSONNEL

Acute Toxicology

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SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6052 when applied to the skin of rabbits.

The study was conducted using three male and three female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group.

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6052	2	3	3
3	T-6052	200	3	3
4	T-6052	1,000	3	3

a Administered at a dose volume of 2.0 mL/kg.

The back of each rabbit was clipped free of hair and a single dose of the respective material at the indicated dose level was administered to the skin of the rabbits. The treatment sites remained intact. The area of application was covered with a gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for a 24-hour exposure period.

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. Body weights were determined on Day -8 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before in-life initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6052 did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared clinically normal throughout the study. No dermal irritation was observed at the dermal scoring intervals as a result of the application of distilled water or T-6052 at any of the dose levels.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of a test material when applied to the skin of rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6052 and described as a clear, colorless liquid. The control material was distilled water and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test and control materials were stored at room temperature. A reserve sample of each test and control material was taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any unused test material was returned to the Sponsor after completion of the in-life phase according to HWI SOP. Any remaining control material is retained for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc., Denver, Pennsylvania on December 28, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -8. The weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. One female animal (No. F53409) was replaced in the study prior to treatment due to poor health. This animal was replaced with another female (No. F52982) which was treated in the same manner.

Study Design

Animals weighing from 2,052 to 2,471 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6052	2	3	3
3	T-6052	200	3	3
4	T-6052	1,000	3	3

a Administered at a dose volume of 2.0 mL/kg.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The intact skin of the test sites was inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped on Days 8 and 29 to aid in visualizing the application sites.

Dose Administration

All animals received a single administration of the respective test or control material. The day of treatment was designated as Day 1.

Group 1. An individual dose (2.0 mL/kg) was calculated and measured based on each animal's body weight on the day of treatment. The control material (distilled water) was applied evenly to the test site at a rate of approximately 0.04 mL/cm^2.

Groups 2, 3, and 4. For the Group 2 animals (2 mg/kg), the test material (T-6052) was mixed with distilled water to a concentration of 200 mg/mL and applied at a dose volume of 0.01 mL/kg. The mixture was stored at room temperature until administered. The test material was administered undiluted to the test sites of the Groups 3 and 4 animals (200 or 1,000 mg/kg, respectively) using the average bulk density of 0.98 g/mL to determine the dose volume for each dose level (0.20 and 1.02 mL/kg, respectively). An individual dose of the respective test material or test material mixture was calculated for each animal based on its body weight on the day of treatment. The area of exposure for the 2, 200, and 1,000 mg/kg dose levels was 4, 25, and 100 cm^2, respectively. The approximate rate of application ranged from 0.006 to 0.024 mL/cm^2.

Each area of application was covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during the 24-hour exposure period.

Approximately 24 hours after test or control material application, the restraining collars and bandages were removed and any residual test material was removed with tap water and disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days.

Body weights were determined for randomization purposes on Day -8, before test material administration (Day 1), and at in-life termination (Day 29).

The initial dermal irritation reading was made before test or control material administration according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8.

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of test and control material application were washed with lukewarm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106), along with their corresponding weights or volumes. The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the tissue samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1. All animals exhibited body weight gains from Day 1 to Day 29.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study.

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6052 at any of the dose levels.

Pathology

Individual animal pathology comments are presented in Table 4. Individual animal tissue weights and bile volumes are in Table 5. There were no lesions observed in any of the animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6052 were evaluated in male and female albino rabbits when administered as a single dermal application. Application of this material did not result in any dermal irritation or test material-related in-life clinical effects. There were no effects on body weight gain or macroscopic findings at necropsy.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-27-95
Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

HWI 6329-135

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from four dose levels euthanized and necropsied at the termination of the study. The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, there were no visible lesions in any of the animals. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. After necropsy, the animals were discarded.

Thomas E. Palmer, PhD
Pathologist

6-27-95
Date

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Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -8	Day 1	Day 29	Animal Number	Randomization Day -8	Day 1	Day 29
Group 1 (Control) - Distilled Water (0 mg/kg)							
F52979	1,903	2,072	2,559	F52976	2,151	2,259	2,681
F52972	2,090	2,362	3,041	F52983	2,116	2,296	2,863
F52973	1,921	2,211	2,818	F52975	2,043	2,265	2,671
Mean	1,971	2,215	2,806		2,103	2,273	2,738
Group 2 - T-6052 (2 mg/kg)							
F52990	2,095	2,351	2,863	F52982[a]	1,885	2,145	2,681
F52997	2,031	2,205	2,928	F52994	2,022	2,261	2,914
F52986	2,034	2,332	2,816	F53410	2,220	2,471	2,875
Mean	2,053	2,296	2,869		2,042	2,292	2,823
Group 3 - T-6052 (200 mg/kg)							
F52996	2,190	2,302	2,897	F52989	2,097	2,316	2,874
F52992	1,889	2,052	2,729	F52993	1,993	2,234	2,651
F52984	1,950	2,257	2,993	F52977	2,049	2,323	2,687
Mean	2,010	2,204	2,873		2,046	2,291	2,737
Group 4 - T-6052 (1,000 mg/kg)							
F52980	1,936	2,184	2,637	F52995	2,131	2,249	2,644
F52978	2,181	2,384	3,063	F52987	2,140	2,274	2,817
F52991	2,108	2,351	3,142	F52988	2,188	2,423	3,015
Mean	2,075	2,306	2,947		2,153	2,315	2,825

a Animal No. F53409 was originally selected by the randomization program for use in the study but was replaced prior to treatment with No. F52982 due to poor health.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 through 29
		Group 1 (Control) - Distilled Water (0 mg/kg)		
Male	F52979	Appeared normal	✓	✓
	F52972	Appeared normal	✓	✓
	F52973	Appeared normal	✓	✓
Female	F52976	Appeared normal	✓	✓
	F52983	Appeared normal	✓	✓
	F52975	Appeared normal	✓	✓
		Group 2 - T-6052 (2 mg/kg)		
Male	F52990	Appeared normal	✓	✓
	F52997	Appeared normal	✓	✓
	F52986	Appeared normal	✓	✓
Female	F52982	Appeared normal	✓	✓
	F52994	Appeared normal	✓	✓
	F53410	Appeared normal	✓	✓

✓ Condition existed.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 through 29
		Group 3 - T-6052 (200 mg/kg)		
Male	F52996	Appeared normal	✓	✓
	F52992	Appeared normal	✓	✓
	F52984	Appeared normal	✓	✓
Female	F52989	Appeared normal	✓	✓
	F52993	Appeared normal	✓	✓
	F52977	Appeared normal	✓	✓
		Group 4 - T-6052 (1,000 mg/kg)		
Male	F52980	Appeared normal	✓	✓
	F52978	Appeared normal	✓	✓
	F52991	Appeared normal	✓	✓
Female	F52995	Appeared normal	✓	✓
	F52987	Appeared normal	✓	✓
	F52988	Appeared normal	✓	✓

✓ Condition existed.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Distilled Water (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52979				Animal No. F52976			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52972				Animal No. F52983			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52973				Animal No. F52975			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6052 (2 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52990				Animal No. F52982			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52997				Animal No. F52994			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52986				Animal No. F53410			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6052 (200 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52996				Animal No. F52989			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52992				Animal No. F52993			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52984				Animal No. F52977			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6052 (1,000 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52980				Animal No. F52995			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52978				Animal No. F52987			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52991				Animal No. F52988			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 1 (Control) - Distilled Water (0 mg/kg)		
F52979	M	-	29	No visible lesions.
F52972	M	-	29	No visible lesions.
F52973	M	-	29	No visible lesions.
F52976	F	-	29	No visible lesions.
F52983	F	-	29	No visible lesions.
F52975	F	-	29	No visible lesions.
		Group 2 - T-6052 (2 mg/kg)		
F52990	M	-	29	No visible lesions.
F52997	M	-	29	No visible lesions.
F52986	M	-	29	No visible lesions.
F52982	F	-	29	No visible lesions.
F52994	F	-	29	No visible lesions.
F53410	F	-	29	No visible lesions.
		Group 3 - T-6052 (200 mg/kg)		
F52996	M	-	29	No visible lesions.
F52992	M	-	29	No visible lesions.
F52984	M	-	29	No visible lesions.
F52989	F	-	29	No visible lesions.
F52993	F	-	29	No visible lesions.
F52977	F	-	29	No visible lesions.

- Not applicable.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 4 - T-6052 (1,000 mg/kg)		
F52980	M	-	29	No visible lesions.
F52978	M	-	29	No visible lesions.
F52991	M	-	29	No visible lesions.
F52995	F	-	29	No visible lesions.
F52987	F	-	29	No visible lesions.
F52988	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F52979	91.387	-	0.593	0.9
	F52972	83.176	16.196	0.358	1.15
	F52973	76.442	-	0.800	1.2
Female	F52976	61.443	-	0.445	0.8
	F52983	98.109	-	0.393	0.9
	F52975	87.984	14.617	0.608	0.4
		Group 2 - T-6052 (2 mg/kg)			
Male	F52990	81.033	-	0.281	0.4
	F52997	92.537	-	0.421	0.5
	F52986	83.745	16.924	0.632	1.1
Female	F52982	84.395	-	0.568	1.1
	F52994	83.650	15.618	0.432	1.4
	F53410	91.541	-	0.386	1.1
		Group 3 - T-6052 (200 mg/kg)			
Male	F52996	78.540	-	0.673	0.46
	F52992	80.845	-	0.511	1.31
	F52984	89.277	15.044	0.421	0.75
Female	F52989	98.425	16.883	0.837	0.7
	F52993	88.925	-	0.848	0.55
	F52977	72.840	-	0.526	0.9

- Not applicable.

Table 5 (Continued)
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 4 - T-6052 (1,000 mg/kg)			
Male	F52980	83.049	15.630	0.896	1.0
	F52978	84.235	-	0.547	1.6
	F52991	88.738	-	0.439	0.9
Female	F52995	63.956	14.923	0.508	0.25
	F52987	82.019	-	0.287	1.35
	F52988	83.911	-	0.875	1.2

- Not applicable.

APPENDIX A

Protocol TP3016.AB
Protocol Amendment No. 1

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6052 in Rabbits

Date:

December 30, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-135

Phone 608 241 4471 Fax 608 241
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of
T-6052 in Rabbits

HWI No.	6329-135
Test Material	T-6052
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	January 5, 1995
Experimental Termination Date	February 2, 1995
Draft Report Date	March 16, 1995

1. Study
Single-Dose Dermal Absorption/Toxicity Study in Rabbits

2. Purpose
To assess the systemic absorption and toxicity and relative skin irritancy of a test material when applied to the skin of rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6052

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test and control materials will be taken for this study.

The test and control material reserve samples will be stored at HWI in a freezer set to maintain a temperature of -20°C ±10°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

F. Retention
Any unused test material will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Distilled water

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this manufactured material is considered to be adequate for the purposes of this study.

D. Storage Conditions
Room temperature

E. Reserve Samples
See Section 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
12 males and 12 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0*	3	3
2	T-6052	2**	3	3
3	T-6052	200	3	3
4	T-6052	1000	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered at a dose volume of .01 mL/kg

(2) Preparation of Exposure Area
On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration
All animals will receive a single administration of the respective test or control material. The day of treatment will be designated as Day 1. The dose for each animal in Group 2 will be diluted with distilled water and applied at a dose volume of .01 mL/kg. The respective dose for each animal in Groups 3 and 4 will be applied undiluted. All doses in Groups 1-4 will be based on the animal's body weight just before administration and will be spread onto the area of exposure in a thin and uniform a layer. The area of application (Groups 1-4) will be covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during the 24-hour application period.

(4) Reason for Route of Administration
The dermal route is a potential route of exposure in humans.

(5) Removal of Test Material
Approximately 24 hours after test or control material application the bandages and collars will be removed and the residual test material will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Clinical Observations
For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days. Observations may be extended when directed by the study director.

(2) Reading of Dermal Irritation
Before test or control material administration the initial dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8. Individual dermal irritation records will be maintained for each animal.

(3) Body Weights
For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day)

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 8, 15, and 22. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29). The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -20°C ±10°C. The separated serum and cellular fractions will be sent frozen on dry ice to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

D. Pathology

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section D. Pathology, (3) Sample Collection, will be collected. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice
At termination of the experimental phase (Day 29), surviving animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded.

(3) Sample Collection
The sites of test and control material application will be washed with lukewarm tap water prior to the necropsy procedure. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necopsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

The tissues (liver, bile, dermal application site, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.C.(4).(c). The Sponsor is responsible for the retention and disposition of the samples.

E. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings/gross pathology report

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Anatomical pathology records
Sample collection records
Shipping records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

1-5-95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

12-30-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12/30/94
Date

(6329-135.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema

0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia

0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation

0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring

0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study
of T-6052 in Rabbits

HWI 6329-135

Sponsor	Contractor
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective January 24, 1995

At the request of the Sponsor, the weights of tissues collected and the volume of bile collected will be documented in the raw data. These weights and volumes will be included with the sample shipment. Modify the following sections of the protocol to include these additions.

1. Page 9, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first and second paragraphs of this section with the following underlined additions:

   The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.

   The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

2. Page 9, 8. Report. Add the following to this section:

   Individual animal tissue weights and bile volumes

Phone 608 241 4471 Fax 608 241 7227
EXPRESS MAIL DELIVERY 3301 KINSMAN BLVD MADISON WI 53704

PROTOCOL AMENDMENT APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M Toxicology Service Medical Department

2/15/95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

2-6-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

2-7-95
Date

(6329-135.Aml.dsk2)

9.1.2 Analytical protocol AMDT-020795.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-135

Study Number: AMDT-020795.1
Test Substance: FC-120 (T-6052)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25,1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson Date
Study Director

John Z. Butenhoff 10/30/95
John Butenhoff, PhD Date
Sponsor Representative

1.0 PURPOSE

This study is designed to provide information as to whether FC-120 (T-6052) is dermally absorbed. The analytical aspect of this study is to determine fluorine-containing compounds in the liver and serum of rabbits. By comparison of the data obtained after dermal absorption with that obtained after intravenous injection, assessment of the extent of dermal absorption can be performed.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

2.1.2 Analytical Reference Matrix: Bovine liver, bovine serum and rabbit serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver, bovine serum and rabbit serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4 liver), Sigma Chemical Company (2.1.2, 2.1.4 bovine serum), AMDT 110394.1 (Hwi#6329-123) control group animals (2.1.2, 2.1.4 rabbit serum)

2.3 Number of Test and Control Samples: Tissues and fluids from 18 test animals and 6 control animals. Tissues and fluids include liver, kidney, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-135), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver. To confirm the identity of fluorine-containing compounds present in liver (if any at 28 days) and serum at various intervals, electrospray mass spectrometry may be selected as one of the analytical techniques employed. Not all of the tissues and fluid samples will be analyzed. When sufficient data has been collected to meet the objectives of the study in the opinion of the Study Director, analysis will cease.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

4.1.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include regression analysis of serum concentrations with time and averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

8.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D Johnson
Group Leader 10/3/95
Date

Carl Kevin Bostwick
Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

James W. Johnson 10-31-95
Group Leader Date

Carl Von Furstenberg 10-31-95
Quality Assurance Date

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 Reagents

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluorooctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step **9.12** of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 Calibration

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 Storage Conditions for Standards

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in **8.3.**
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 Quality Control - not applicable
10.2 Precision and Accuracy- not applicable
10.3 Other Validation Parameters- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W_____	FC-95 approx 1 ppm actual ppm #W_____	FC-95 approx. 5 ppm actual ppm #W_____	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
[1] study number where the original worksheet is located and place a copy.					

Liver Extraction Process:	Date & Initials
Pipet 1 mL of Liver Solution	
Pipet 100 uL of 12 ppm Internal Standard	Std. #
Vortex 15 sec.	
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	
Pipet 5 mL of Ethyl Acetate	
Vortex 20-30 sec.	
Shake 20 min.	
Centrifuge 20-25 min.	
Remove a 4 mL aliquot of organic layer	
Blow down to near dryness (≤0.25 mL) with N_2	
Add 1 mL of 1:1 Acetonitrile/H_2O	TN#
Vortex 15 sec.	
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.	

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES$^-$.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES$^-$, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 Using the Autosampler

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- **9.3.3.1** Push the sample button
- **9.3.3.2** Set sample loop size = 100 uL
- **9.3.3.3** Set inject/sample = 2
- **9.3.3.4** Set Cycle time = 0
- **9.3.3.5** Name the file: Livers
- **9.3.3.6** Identify the tray used
- **9.3.3.7** Add the samples to Queue by pressing "Enter"
- **9.3.3.8** Press "Run" to start

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

Revision Number: 0 **Revision Date:** None

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Kevin Burkett 9-27-95
Quality Assurance Date

Software: IBM MS Word, 6.0
Affected Documents: **AMDT-EP-26,** Operation and Maintenance of the Skalar Segmented Flow Analyzer

1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps
4.1.2 Autopipets, oxford or equivalent with plastic tips
4.1.3 Polypropylene volumetric flasks, 100 mL
4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.
4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent
4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.
4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.
4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.
4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.
4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Prepare calibration standards as in section 4.3.

8.2 Calibration

8.2.1 The standards are analyzed at the beginning of the run.

8.3 Storage Conditions for Standards

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 Start Up Procedure

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.
9.1.2 Put the fluoride electrodes in the electrode chamber.
9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.
9.1.4 Put the reagent-lines in the appropriate bottles.
9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**
9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 Starting a Run

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.
9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.
9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.
9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.
9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under **9.2.1** (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-M-1, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Serum

Method Identification Number: AMDT-M-14 **Adoption Date:** 10-3-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

James D Johnson 10/3/95
Group Leader Date

Eli Von Benkitz 9-27-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological fluids, particularly serum.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 100 µl. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are to be handled with plastic pipettes. A new pipette is to be used for each sample.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit serum studies, use beef serum as the matrix.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked serum curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef serum as the matrix.
8.3.2.3 Put serum blank in Dohrmann boat. Combust sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.
8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 If beef serum is frozen, thaw at least enough to complete the standard curve analysis for the day (≈30 mL).
8.3.3.2 Pipette 100µL of beef serum into Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef serum.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.
9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and pipette 100µL of sample into the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.

9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 1.5 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-WATER program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator and/or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control

10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

9.1.3 Amendment to Analytical Protocol AMDT-020795.1

Attachment I

GLP Study

Protocol Amendment

Study Number: AMDT-020795.1

Study Title: Single-Dose Dermal Absorption /Toxicity Study of T-6052 in Rabbits

Study Director: James D. Johnson

Amendment Date: November 8, 1995 Amendment Number: 1

This amendment modifies the following portion of the protocol:

AMDT-M-14-0 specifies using bovine serum for the blanks in the thermal extraction. However, rabbit serum from the control animals (AMDT-110394.1) was used because the bovine serum blanks were higher than the samples.

Approved by:

James D Johnson 11/20/95

Study Director Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor Original to: Study Director Copies to: QAU Files

Study Title: **Single-dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits**

Study Number: AMDT-020795.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/13/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95
QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:

Jim Johnson
Dave Christenson

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:

Jim Johnson
Rich Youngblom

Quality Assurance Unit:

Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-020795.1, HWI 6329-135
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group**

	ug	Std. Dev.
Control Group	16.7 ±	5.9
2.0 mg/kg dose (T6052)	12.6 ±	3.2
200 mg/kg dose (T6052)	18.3* ±	4.2
1000 mg/kg dose (T6052)	24.1 ±	4.2

** Calculated as the mean of triplicate samples from each of three male and three female rabbits.
* One outlier omitted. Value of re-analyzed sample included in this report.

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
Liver Blk-1		0.133		0.112			
Liver Blk-2		0.108		0.124			
Liver Spk-1	80%	1.10		0.110			
Liver Spk-2	90%	0.980		0.139			
Liver Spk-3	92%	1.06		0.132			
Liver Spk-4	94%	2.72		0.105			
Liver Spk-5	108%	3.24		0.101			
Liver Spk-6	94%	2.43		0.117			
Liver blank-3		0.393		0.119			
Liver blank-4		0.320		0.121			
F52972-1		0.360		0.107	82.5		
F52972-2		0.317	0.331	0.103	82.5	27.3	0.0
F52972-3		0.315		0.132	82.5		
F52973-1		0.240		0.148	75.8		
F52973-2		0.243	0.251	0.150	75.8	19.0	0.0
F52973-3		0.269		0.131	75.8		
F52979-1		0.214		0.119	90.6		
F52979-2		0.171	0.183	0.148	90.6	16.6	0.0
F52979-3		0.166		0.133	90.6		
Liver Blank-1		0.276		0.114			
Liver Blank-2		0.131		0.135			
Liver Spike-1	80%	0.864		0.141			
Liver Spike-2	89%	1.35		0.100			
Liver Spike-3	79%	0.863		0.138			
Liver Spike-4	77%	0.965		0.121			
Liver Blank-A		0.000		0.132			
Liver Spike-5	88%	1.02		0.130			
Liver Spike-6	90%	0.993		0.138			
Liver Spike-7	92%	1.04		0.134			
F52975-1		0.165		0.136	86.4		
F52975-2		0.135	0.138	0.135	86.4	12.0	0.0
F52975-3		0.116		0.139	86.4		
F52976-1		0.135		0.150	60.8		
F52976-2		0.125	0.185	0.141	60.8	11.2	0.0
F52976-3		0.294		0.127	60.8		
F52983-1		0.153		0.134	96.5		
F52983-2		0.151	0.145	0.116	96.5	14.0	0.0
F52983-3		0.132		0.128	96.5		
F52986-1		0.149		0.125	82.8		
F52986-2		0.147	0.147	0.108	82.8	12.2	2.0
F52986-3		0.145		0.135	82.8		
F52990-1		0.162		0.145	80.4		
F52990-2		0.196	0.184	0.120	80.4	14.8	2.0
F52990-3		0.195		0.107	80.4		
F52997-1		0.220		0.114	91.7		
F52997-2		0.243	0.195	0.116	91.7	17.9	2.0
F52997-3		0.123		0.150	91.7		

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)	
F52982-1		0.136		0.147	82.4			
F52982-2		0.153	0.134	0.105	82.4	11.0	2.0	
F52982-3		0.113		0.116	82.4			
F52994-1		0.121		0.130	82.9			
F52994-2		0.105	0.113	0.151	82.9	9.37	2.0	
F52994-3		0.113		0.148	82.9			
F53410-1		0.114		0.129	91.0			
F53410-2		0.130	0.115	0.119	91.0	10.5	2.0	
F53410-3		0.102		0.146	91.0			
Liver Blank-1		0.207		0.106				
Liver Blank-2		0.130		0.102				
Liver Spike-1	65%	0.911		0.108				
Liver Spike-2	65%	0.747		0.131				
Liver Spike-3	71%	0.751		0.144				
Liver Spike-4	85%	1.02		0.125				
Liver Spike-5	96%	1.13		0.129				
Liver Spike-6	90%	1.04		0.131				
Liver Spike-7	80%	0.946		0.128				
F52984-1		0.216		0.112	88.5			
F52984-2		0.224	0.200	0.104	88.5	17.7	200	
F52984-3		0.160		0.131	88.5			
F52992-1		0.173		0.121	80.6			
F52992-2		0.205	0.209	0.115	80.6	16.8	200	
F52992-3		0.247		0.136	80.6			
F52996-1		0.843		0.134	77.8			*Sample
F52996-2		0.272	1.06	0.115	77.8	82.5*	200	reanalyzed
F52996-3		2.07		0.142	77.8			below
F52977-1		0.207		0.140	72.0			
F52977-2		0.180	0.187	0.126	72.0	13.5	200	
F52977-3		0.173		0.128	72.0			
F52989-1		0.217		0.106	97.9			
F52989-2		0.249	0.219	0.134	97.9	21.5	200	
F52989-3		0.192		0.135	97.9			
F52993-1		0.174		0.141	88.3			
F52993-2		0.199	0.175	0.129	88.3	15.5	200	
F52993-3		0.153		0.147	88.3			
liver blank-1		0.312		0.148				
liver spike-1	72%	0.741		0.146				
liver spike-2	75%	0.928		0.123				
liver spike-3	67%	0.670		0.151				
liver spike-4	77%	0.826		0.141				
liver spike-5	77%	0.934		0.124				
liver spike-6	88%	1.27		0.105				
liver spike-7	152%	1.52		0.151				
liver spike-8	81%	0.829		0.148				
liver spike-9	88%	1.32		0.101				
liver spike-10	88%	1.12		0.118				

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)	
F52996-1		0.291		0.136	77.8			**Repeat
F52996-2		0.244	0.319	0.141	77.8	24.8**	200	of above
F52996-3		0.422		0.103	77.8			analysis
F52978-1		0.259		0.148	83.2			
F52978-2		0.224	0.236	0.141	83.2	19.6	1000	
F52978-3		0.225		0.143	83.2			
Blank liver 1		0.411		0.129				
Blank liver 2		4.45		0.146				
Blank liver 3		2.01		0.149				
Blank liver 4		0.340		0.137				
Blank liver 5		0.277		0.145				
Blank liver 6		0.404		0.114				
Liver Spike-1	130%	1.37		0.143				
Liver Spike-2	84%	1.09		0.117				
Liver Spike-3	87%	0.831		0.158				
Liver Spike-4	82%	0.955		0.130				
Liver Spike-5	77%	0.784		0.150				
F52980-1		0.289		0.133	82.0			
F52980-2		0.235	0.252	0.148	82.0	20.7	1000	
F52980-3		0.231		0.158	82.0			
F52991-1		0.330		0.134	86.9			
F52991-2		0.270	0.279	0.147	86.9	24.3	1000	
F52991-3		0.238		0.120	86.9			
F52987-1		0.204		0.122	81.2			
F52987-2		0.604	0.346	0.150	81.2	28.0	1000	
F52987-3		0.230		0.150	81.2			
F52988-1		0.249		0.118	83.2			
F52988-2		0.490	0.360	0.138	83.2	29.9	1000	
F52988-3		0.340		0.109	83.2			
F52995-1		0.453		0.147	63.1			
F52995-2		0.315	0.345	0.136	63.1	21.8	1000	
F52995-3		0.266		0.113	63.1			
liver blank-1		0.168		0.146				
liver spike-1	72%	0.862		0.126				
liver spike-2	81%	0.820		0.150				
liver spike-3	82%	0.847		0.146				
liver spike-4	77%	0.905		0.129				
liver spike-5	83%	2.03		0.123				
liver spike-6	84%	1.89		0.135				
liver spike-7	81%	1.75		0.141				
liver blank-2		0.266		0.131				

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

A-
Containing page
A-1 thru A-
11/9/95
DLC

HWI # 6329-135

Study:	Single-Dose Dermal Absorption
Protocol Number:	TP3016.AB
Test Material:	T-6052 in Rabbits (FC-120)
Matrix:	Liver
R Squared Value:	Screening
Response Factor Amount:	N/A
Analyst:	DLC
Date:	4/6/95
Method:	
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE File:	040695B

DLC 11-9-95

Rabbit liver extracts screened for M⁻ 599 ion only, no quantitation performed.

Group Dose	Sample #	Ion Count Area *	Extracted wt g	Dilution factor	Concentration µg/g ****	Total mass of liver g	Total amount of FC-95 per liver mg	% of FC-95
Group 1: **0 mg/kg ****	F52975	N.D.						
	F52976	re-extract						
	F52983	re-extract						
	F52972	N.D.						
	F52973	N.D.						
	F52979	N.D.						
Group 2: **2 mg /kg *****	F52986	N.D.						
	F52990	N.D.						
	F52997	re-extract						
	F52982	N.D.						
	F52994	N.D.						
	F53410	N.D.						
Group 3: **200 mg/kg *****	F52984	$						
	F52992	$						
	F52996	re-extract						
	F52977	$						
	F52989	N.D.						
	F52993	N.D.						
Group 4: **1000 mg/kg *****	F52978	$						
	F52980	$						
	F52991	$						
	F52987	$						
	F52988	$						
	F52995	re-extract						

* SIR Monitoring of M-599 and 598.

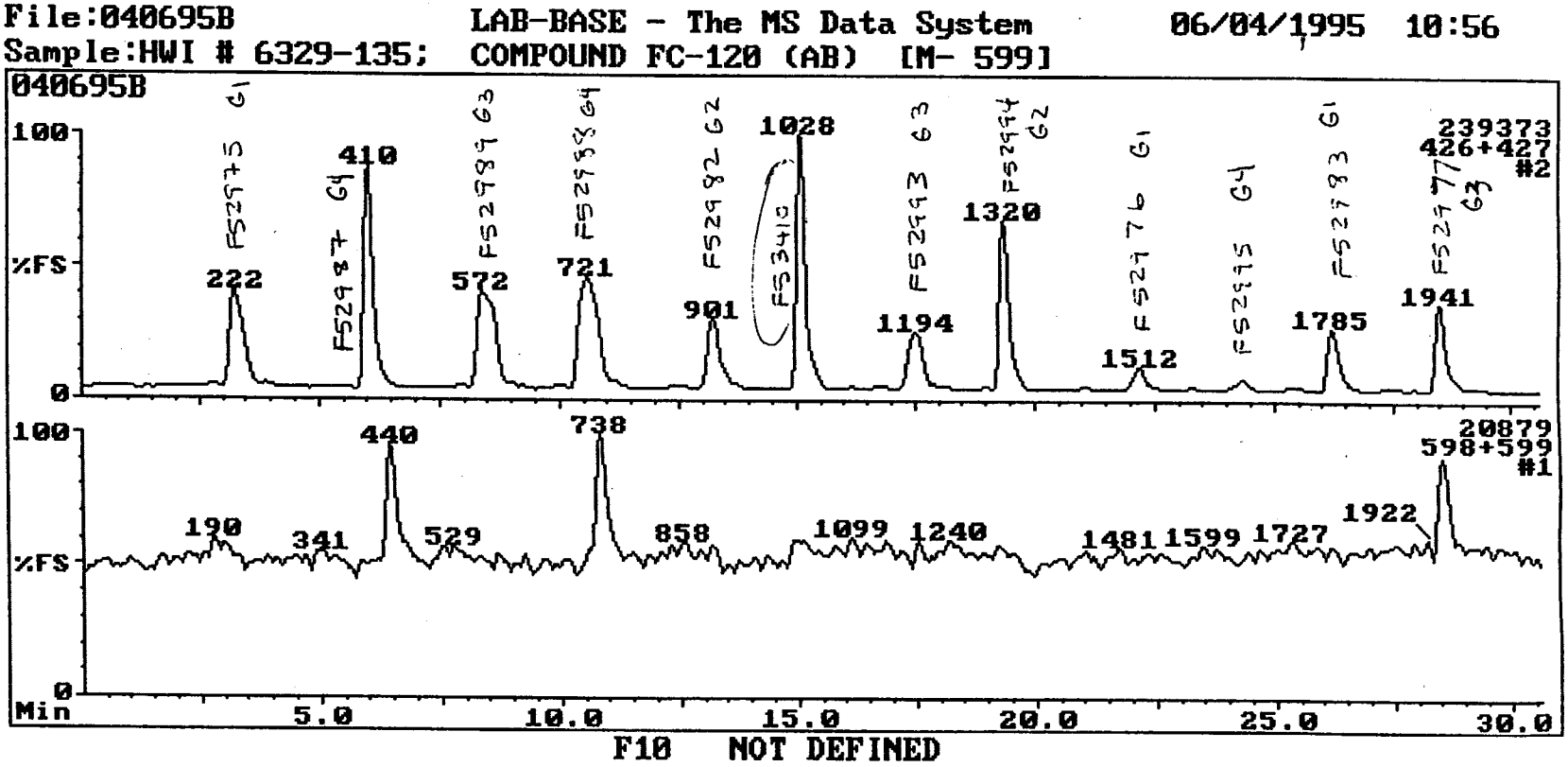
$ = Positive response for ion monitored.

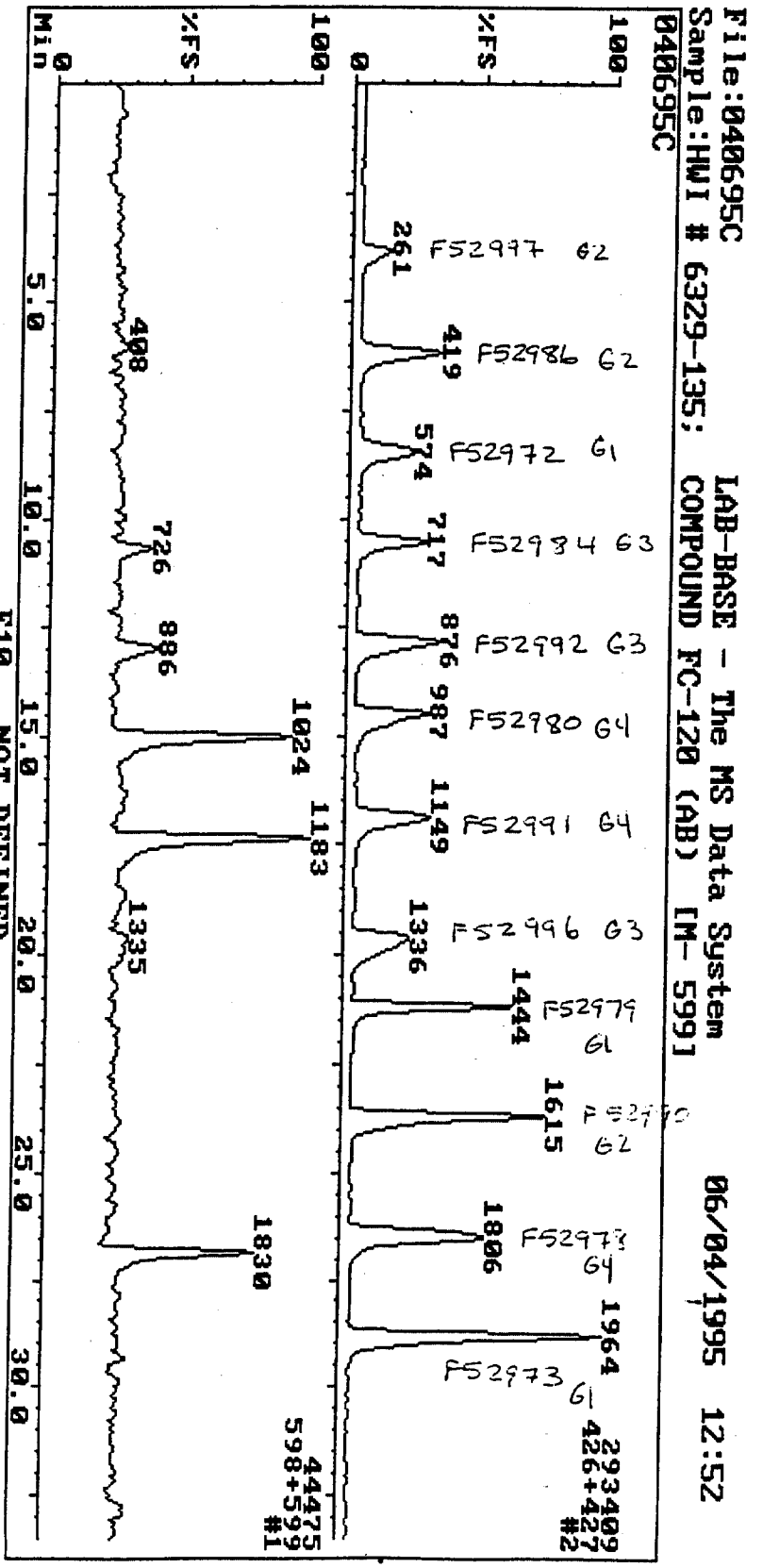
** Administered at a dose volume of 2.0 mL/kg.

*** Administered at a dose volume of 0.01 mL/kg.

****The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

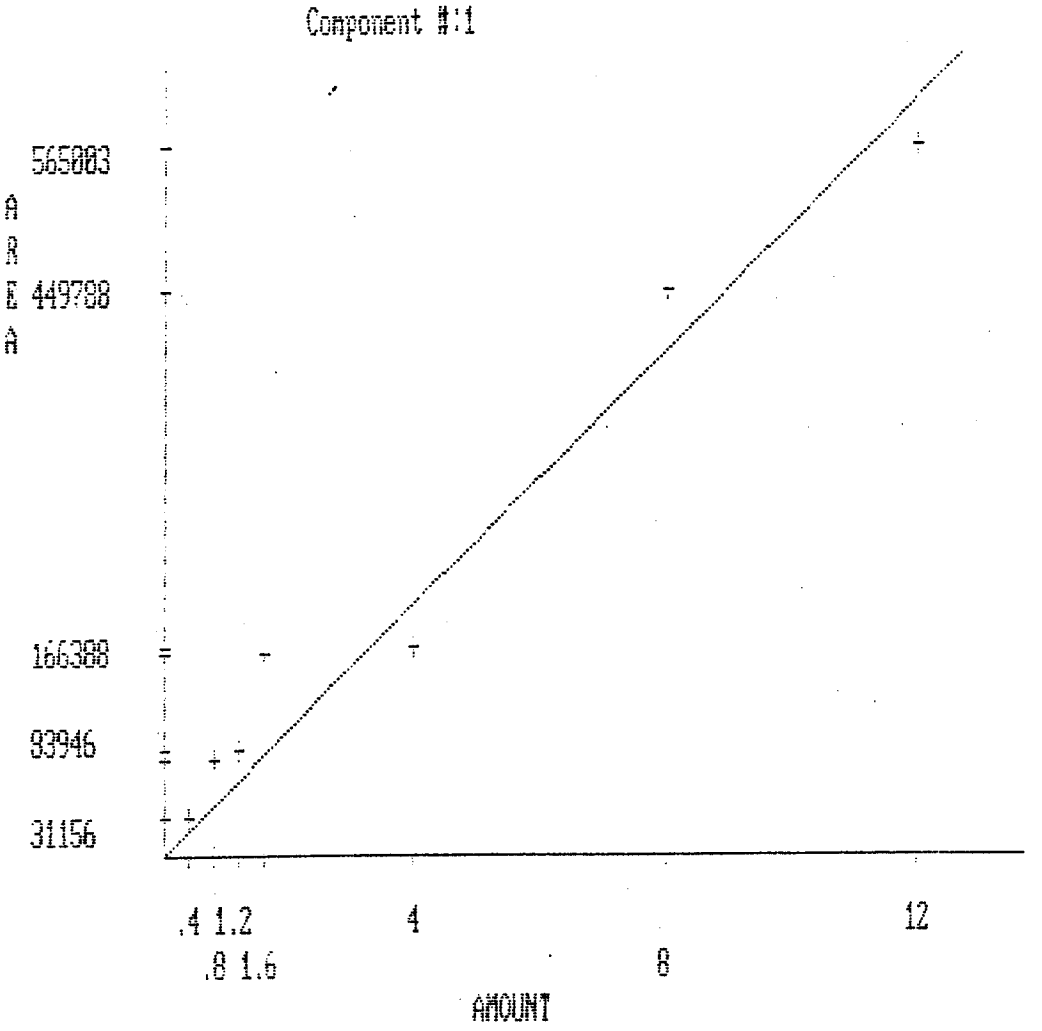
Electrospray Chromatogram
from LABBase file: 040695B
Study # 6329-135
SIR: 599
negative ion
10-19-95 DLC





LAB BASE
FILE 040395C
HWE* 6329-135
5/8/95
DLC

Method DLCLIV
Sample DLCLIV
Operator DLC
Run date 05-08-1995 11:39:38 Version: 8
Printed on 05-08-1995 AT 11:39:58
Straight Line Fit forced through Origin.



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	31156
2	0.8000	77583
3	1.2000	83946
4	1.6000	160773
5	4.0000	166388
6	8.0000	449788
7	12.0000	565003

Y = SLOPE * X + INTERCEPT

Area = 5.0159E+04 * Amount + 0.0000E+00
Amount = 1.9937E-05 * Area + 0.0000E+00
R squared = 0.9467

9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

HWI 6329-135
AMDT 011095.1
Dohrmann Serum Analysis
Analysis Dates: 08/02/95 - 08/18/95

All serum samples were thermally extracted by a modified Dohrmann DX2000 Organic Halide Analyzer and collected in a 1:1 milli Q water and TISAB solution. The samples were measured on an Orion EA940 expandable ion analyzer. The Dohrmann was calibrated using 34ppm, 40ppm, 62ppm, 100ppm, 124ppm, 250ppm, and 500ppm FC-95 standards for serum curve 1. The same FC-95 standards were used to calibrate the Dohrmann with the exception of 250ppm and 500ppm for serum curve 2. The Orion was calibrated by direct measurement with no blank correction using 0.05ppm, 0.1ppm, 0.5ppm, 1.0ppm and 1.5ppm F^- standards. The slope, intercept, and correlation were recorded in the appropriate logbook.

A summary table is included, showing the ppm F^- in each sample (see page 2). An initial calibration curve with standard deviation, %RSD, R^2 value and equation of the line is on pages 8 - 11.

Pages 3 - 7 show the excel spreadsheet that was generated when the samples were being analyzed. The Dohrmann FC-95 initial calibration curve was not used to generate the data in the spreadsheet. Any Orion reading below 0.05 is below the Orion calibration and should be considered an estimate.

The FC-95 initial calibration curve 1 was spiked into bovine serum. Bovine serum was used as blanks and QC check samples on 08/02/95 and 08/03/95. However, due to problems with blanks having higher readings than the samples, serum curve 2 was analyzed using rabbit serum from study # 6329-123 rabbit # F52346. On days 08/15/95 - 08/18/95 rabbit serum from study # 6329-123 rabbits F52346, F52335, and F52332, were used for blanks and QC check samples

We were unable to located serum samples for Day 2 through Day 4. This study was discontinued after day 8 because nothing was found in the first day of sampling.

Dean K. Plummer

FC 120 AB

HWI 6329-135

Fluoride concentration in rabbit serum (ppm F-)

Group 1
Dosage: 0 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52972	0.533	0.311	0.553	0.582
F52973	0.498	0.178	0.859	0.712
F52979	0.551	0.490	0.612	0.561
F52975	0.517	0.436	0.674	0.524
F52976	0.479	0.323	0.501	0.472
F52983	0.506	0.286	0.503	0.677

Group 2
Dosage: 2 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52986	1.45	0.338	0.393	0.610
F52990	0.650	0.277	0.463	0.466
F52997	0.444	0.305	0.36	0.529
F52982	0.466	0.283	0.298	0.438
F52994	0.446	1.27	0.346	0.620
F53410	0.388	0.697	0.354	0.677

Group 3
Dosage: 200 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52984	0.594	0.694	0.271	0.620
F52992	0.550	0.526	0.281	0.636
F52996	0.568	0.845	0.257	0.712
F52977	0.602	1.10	0.274	0.685
F52989	0.642	1.57	0.641	0.814
F52993	0.642	0.612	0.777	0.580

Group 4
Dosage: 1000 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52978	0.762	0.449	0.777	0.449
F52980	1.01	0.566	0.807	0.516
F52991	1.07	0.709	0.752	0.581
F52987	0.886	0.630	0.700	0.335
F52988	0.549	0.478	0.668	0.249
F52995	0.491	0.513	0.57	0.275

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
BLANK	0.0613	0.1	2.0				1.23		0.123
BLANK	0.0396	0.1	2.0				0.792		0.0792
BLANK	0.0383	0.1	2.0				0.766		0.0766
BLANK	0.0412	0.1	2.0				0.824		0.0824
BLANK	0.0319	0.1	2.0				0.638		0.0638
62-PPM-1	0.0786	0.1	2.0	0.004	62	106%	1.57	0.149	0.157
62-PPM-2	0.0934	0.1	2.0	0.004	62	125%	1.87	0.149	0.187
250-PPM-1	0.304	0.1	2.0	0.004	250	101%	6.08	0.600	0.608
250-PPM-2	0.269	0.1	2.0	0.004	250	90%	5.38	0.600	0.538
BLANK	0.0903	0.1	2.0				1.81		0.181
BLANK	0.0527	0.1	2.0				1.05		0.105
BLANK	0.0326	0.1	2.0				0.652		0.0652
F52986-DAY1	0.0723	0.1	2.0				1.45		0.145
F52990-DAY1	0.0325	0.1	2.0				0.650		0.0650
F52997-DAY1	0.0222	0.1	2.0				0.444		0.0444
F52982-DAY1	0.0233	0.1	2.0				0.466		0.0466
F52994-DAY1	0.0223	0.1	2.0				0.446		0.0446
F53410-DAY1	0.0194	0.1	2.0				0.388		0.0388
62-PPM-1	0.0760	0.1	2.0	0.004	62	102%	1.52	0.149	0.152
250-PPM-1	0.246	0.1	2.0	0.004	250	82%	4.92	0.600	0.492
SERUM BLANK-1	0.207	0.1	2.0				4.13		0.413
SERUM BLANK-2	0.176	0.1	2.0				3.52		0.352
SERUM BLANK-3	0.107	0.1	2.0				2.15		0.215
SERUM BLANK-4	0.0842	0.1	2.0				1.68		0.168
SERUM BLANK-5	0.0745	0.1	2.0				1.49		0.149
SERUM BLANK-6	0.0856	0.1	2.0				1.71		0.171
SERUM BLANK-7	0.0755	0.1	2.0				1.51		0.151
SERUM BLANK-8	0.0691	0.1	2.0				1.38		0.138
SERUM BLANK-9	0.0615	0.1	2.0				1.23		0.123
SERUM BLANK-10	0.0657	0.1	2.0				1.31		0.131
SERUM BLANK-11	0.0592	0.1	2.0				1.18		0.118
SERUM BLANK-12	0.0672	0.1	2.0				1.34		0.134
SERUM BLANK-13	0.0703	0.1	2.0				1.41		0.141
SERUM BLANK-14	0.0674	0.1	2.0				1.35		0.135
SERUM BLANK-15	0.0655	0.1	2.0				1.31		0.131
SERUM BLANK-16	0.0507	0.1	2.0				1.01		0.101
SERUM BLANK-17	0.0434	0.1	2.0				0.868		0.0868
SERUM BLANK-18	0.0403	0.1	2.0				0.806		0.0806
SERUM BLANK-19	0.0398	0.1	2.0				0.796		0.0796
SERUM BLANK-20	0.0375	0.1	2.0				0.751		0.0751
62PPM-1	0.0969	0.1	2.0	0.004	62	130%	1.94	0.149	0.194
62PPM-2	0.0902	0.1	2.0	0.004	62	121%	1.80	0.149	0.180
62PPM-3	0.0869	0.1	2.0	0.004	62	117%	1.74	0.149	0.174
250PPM-1	0.262	0.1	2.0	0.004	250	87%	5.24	0.600	0.524
250PPM-2	0.270	0.1	2.0	0.004	250	90%	5.40	0.600	0.540
F52984DAY1	0.0297	0.1	2.0				0.594		0.0594

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
F52992DAY1	0.0275	0.1	2.0				0.550		0.0550
F52996DAY1	0.0284	0.1	2.0				0.568		0.0568
F52977DAY1	0.0301	0.1	2.0				0.602		0.0602
F52989DAY1	0.0321	0.1	2.0				0.642		0.0642
F52993DAY1	0.0321	0.1	2.0				0.642		0.0642
F52978DAY1	0.0381	0.1	2.0				0.762		0.0762
F52980DAY1	0.0507	0.1	2.0				1.01		0.101
F52991DAY1	0.0533	0.1	2.0				1.07		0.107
F52987DAY1	0.0443	0.1	2.0				0.886		0.0886
62PPM-1	0.0890	0.1	2.0	0.004	62	120%	1.78	0.149	0.178
250PPM-1	0.264	0.1	2.0	0.004	250	88%	5.28	0.600	0.528
8/15/95									
Blank Serum-1	0.0401	0.1	2.0				0.803		0.0803
Blank Serum-2	0.0365	0.1	2.0				0.731		0.0731
Blank Serum-3	0.0279	0.1	2.0				0.558		0.0558
F52988-day1	0.0275	0.1	2.0				0.549		0.0549
F52995-day1	0.0246	0.1	2.0				0.491		0.0491
F52972-day1	0.0266	0.1	2.0				0.533		0.0533
F52973-day1	0.0249	0.1	2.0				0.498		0.0498
F52979-day1	0.0276	0.1	2.0				0.551		0.0551
F52975-day1	0.0259	0.1	2.0				0.517		0.0517
F52976-day1	0.0240	0.1	2.0				0.479		0.0479
F52983-day1	0.0253	0.1	2.0				0.506		0.0506
40ppm-1	0.0388	0.1	2.0	0.004	40	81%	0.776	0.096	0.0776
100ppm-1	0.0982	0.1	2.0	0.004	100	82%	1.96	0.240	0.196
serum blank	0.0201	0.1	2.0				0.403		0.0403
serum blank	0.0192	0.1	2.0				0.383		0.0383
serum blank	0.0147	0.1	2.0				0.294		0.0294
spike 34-1	0.0431	0.1	2.0	0.004	40	90%	0.863	0.096	0.0863
spike 34-2	0.0435	0.1	2.0	0.004	40	91%	0.870	0.096	0.087
spike 100-1	0.0928	0.1	2.0	0.004	100	77%	1.86	0.240	0.186
spike 100-2	0.0905	0.1	2.0	0.004	100	75%	1.81	0.240	0.181
spike 100-3	0.0984	0.1	2.0	0.004	100	82%	1.97	0.240	0.197
F52972 DAY 8	0.0155	0.1	2.0				0.311		0.0311
F52973 DAY 8	0.00864	0.1	2.0				0.173		0.0173
F52979 DAY 8	0.0245	0.1	2.0				0.490		0.0490
F52975 DAY 8	0.0218	0.1	2.0				0.436		0.0436
F52976 DAY 8	0.0161	0.1	2.0				0.323		0.0323
F52983 DAY 8	0.0143	0.1	2.0				0.286		0.0286
F52986 DAY 8	0.0169	0.1	2.0				0.338		0.0338
F52990 DAY 8	0.0139	0.1	2.0				0.277		0.0277
F52997 DAY 8	0.0153	0.1	2.0				0.305		0.0305
F52982 DAY 8	0.0142	0.1	2.0				0.283		0.0283
SPIKE 62-1	0.0343	0.1	2.0	0.004	62	46%	0.685	0.149	0.0685
SPIKE 62-2	0.0853	0.1	2.0	0.004	62	115%	1.71	0.149	0.171
SPIKE124-1	0.106	0.1	2.0	0.004	124	71%	2.11	0.298	0.211

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
SPIKE124-2	0.134	0.1	2.0	0.004	124	90%	2.67	0.298	0.267
Blank	0.0722	0.1	2.0				1.44		0.144
F52994-day 8	0.0634	0.1	2.0				1.27		0.127
F53410 day 8	0.0348	0.1	2.0				0.697		0.0697
F52984 day8	0.0347	0.1	2.0				0.694		0.0694
F52992 day 8	0.0263	0.1	2.0				0.526		0.0526
F52996 day 8	0.0422	0.1	2.0				0.845		0.0845
F52977 day 8	0.0550	0.1	2.0				1.10		0.110
F52989 day 8	0.0407	0.1	2.0				0.814		0.0814
F52993 day 8	0.0306	0.1	2.0				0.612		0.0612
F52978 day 8	0.0272	0.1	2.0				0.544		0.0544
F52980 day 8	0.0283	0.1	2.0				0.566		0.0566
40ppm-1	0.0530	0.1	2.0	0.004	40	110%	1.06	0.096	0.106
124ppm-1	0.124	0.1	2.0	0.004	124	84%	2.49	0.298	0.249
Blank	0.0608	0.1	2.0				1.22		0.122
F52991 day 8	0.0354	0.1	2.0				0.709		0.0709
F52987 day 8	0.0315	0.1	2.0				0.630		0.0630
F52988 day 8	0.0239	0.1	2.0				0.478		0.0478
F52995 day 8	0.0257	0.1	2.0				0.513		0.0513
F52972 day 15	0.0277	0.1	2.0				0.553		0.0553
F52973 day 15	0.0430	0.1	2.0				0.859		0.0859
F52979 day 15	0.0306	0.1	2.0				0.612		0.0612
F52975 day 15	0.0337	0.1	2.0				0.674		0.0674
F52976 day 15	0.0250	0.1	2.0				0.501		0.0501
F52983 day 15	0.0251	0.1	2.0				0.503		0.0503
40 ppm-1	0.0452	0.1	2.0	0.004	40	94%	0.903	0.096	0.0903
124 ppm-1	0.123	0.1	2.0	0.004	124	83%	2.47	0.298	0.247
serum blank	0.0259	0.1	2.0				0.518		0.0518
serum blank	0.0199	0.1	2.0				0.398		0.0398
spike 40-1	0.0373	0.1	2.0	0.004	40	78%	0.745	0.096	0.0745
spike 40-2	0.0390	0.1	2.0	0.004	40	81%	0.781	0.096	0.0781
spike 40-3	0.0448	0.1	2.0	0.004	40	93%	0.896	0.096	0.0896
spike 124-1	0.122	0.1	2.0	0.004	124	82%	2.45	0.298	0.245
spike 124-2	0.134	0.1	2.0	0.004	124	90%	2.68	0.298	0.268
spike 124-3	0.116	0.1	2.0	0.004	124	78%	2.33	0.298	0.233
serum blank	0.0302	0.1	2.0				0.605		0.0605
F52986 DAY15	0.0196	0.1	2.0				0.393		0.0393
F52990 DAY15	0.0231	0.1	2.0				0.463		0.0463
F52997 DAY15	0.0180	0.1	2.0				0.360		0.0360
F52982 DAY15	0.0149	0.1	2.0				0.298		0.0298
F52994 DAY15	0.0173	0.1	2.0				0.346		0.0346
F53410 DAY15	0.0177	0.1	2.0				0.354		0.0354
F52984 DAY15	0.0135	0.1	2.0				0.271		0.0271
F52992 DAY15	0.0141	0.1	2.0				0.281		0.0281
F52996 DAY15	0.0129	0.1	2.0				0.257		0.0257
F52977 DAY15	0.0137	0.1	2.0				0.274		0.0274

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
SPIKE 40-1	0.0373	0.1	2.0	0.004	40	78%	0.746	0.096	0.0746
SPIKE 124-1	0.152	0.1	2.0	0.004	124	102%	3.04	0.298	0.304
serum blank	0.0465	0.1	2.0				0.931		0.0931
F52989 day15	0.0785	0.1	2.0				1.57		0.157
F52993 day 15	0.0389	0.1	2.0				0.777		0.0777
F52978 day 15	0.0389	0.1	2.0				0.777		0.0777
F52980 day 15	0.0404	0.1	2.0				0.807		0.0807
F52991 day 15	0.0376	0.1	2.0				0.752		0.0752
F52987 day 15	0.0350	0.1	2.0				0.700		0.0700
F52988 day 15	0.0334	0.1	2.0				0.668		0.0668
F52995 day 15	0.0285	0.1	2.0				0.570		0.0570
F52972 day 22	0.0291	0.1	2.0				0.582		0.0582
F52973 day 22	0.0356	0.1	2.0				0.712		0.0712
40ppm spike-1	0.0722	0.1	2.0	0.004	40	150%	1.44	0.096	0.144
40ppm spike-2	0.0460	0.1	2.0	0.004	40	96%	0.919	0.096	0.0919
124ppm spike-1	0.110	0.1	2.0	0.004	124	74%	2.20	0.298	0.220
F52979 day 22	0.0280	0.1	2.0				0.561		0.0561
F52975 day 22	0.0262	0.1	2.0				0.524		0.0524
F52976 day 22	0.0236	0.1	2.0				0.472		0.0472
F52983 day 22	0.0339	0.1	2.0				0.677		0.0677
F52986 day 22	0.0305	0.1	2.0				0.610		0.0610
F52990 day 22	0.0233	0.1	2.0				0.466		0.0466
F52997 day 22	0.0264	0.1	2.0				0.529		0.0529
F52982 day 22	0.0219	0.1	2.0				0.438		0.0438
F52994 day 22	0.0310	0.1	2.0				0.620		0.0620
F53410 day 22	0.0339	0.1	2.0				0.677		0.0677
40ppm spike-1	0.0645	0.1	2.0	0.004	40	134%	1.29	0.096	0.129
124 ppm spike -1	0.130	0.1	2.0	0.004	124	88%	2.61	0.298	0.261
62ppm spike-1	0.0898	0.1	2.0	0.004	62	121%	1.80	0.149	0.180
serum blank	0.0405	0.1	2.0				0.810		0.0810
F52984 day 22	0.0310	0.1	2.0				0.620		0.0620
F52992 day 22	0.0318	0.1	2.0				0.636		0.0636
F52996 day 22	0.0356	0.1	2.0				0.712		0.0712
F52977 day 22	0.0343	0.1	2.0				0.685		0.0685
F52989 day 22	0.0321	0.1	2.0				0.641		0.0641
F52993 day 22	0.0290	0.1	2.0				0.580		0.0580
40ppm spike-1	0.0616	0.1	2.0	0.004	40	128%	1.23	0.096	0.123
124 ppm spike -1	0.116	0.1	2.0	0.004	124	78%	2.32	0.298	0.232
serum blank	0.0221	0.1	2.0				0.442		0.0442
serum blank	0.0172	0.1	2.0				0.344		0.0344
spike 40-1	0.0235	0.1	2.0	0.004	40	49%	0.471	0.096	0.0471
spike 40-2	0.0333	0.1	2.0	0.004	40	69%	0.666	0.096	0.0666
spike 40-3	0.0343	0.1	2.0	0.004	40	71%	0.686	0.096	0.0686
spike 40-4	0.0371	0.1	2.0	0.004	40	77%	0.743	0.096	0.0743
spike 124-1	0.0562	0.1	2.0	0.004	124	38%	1.12	0.298	0.112
spike 124-2	0.0910	0.1	2.0	0.004	124	61%	1.82	0.298	0.182

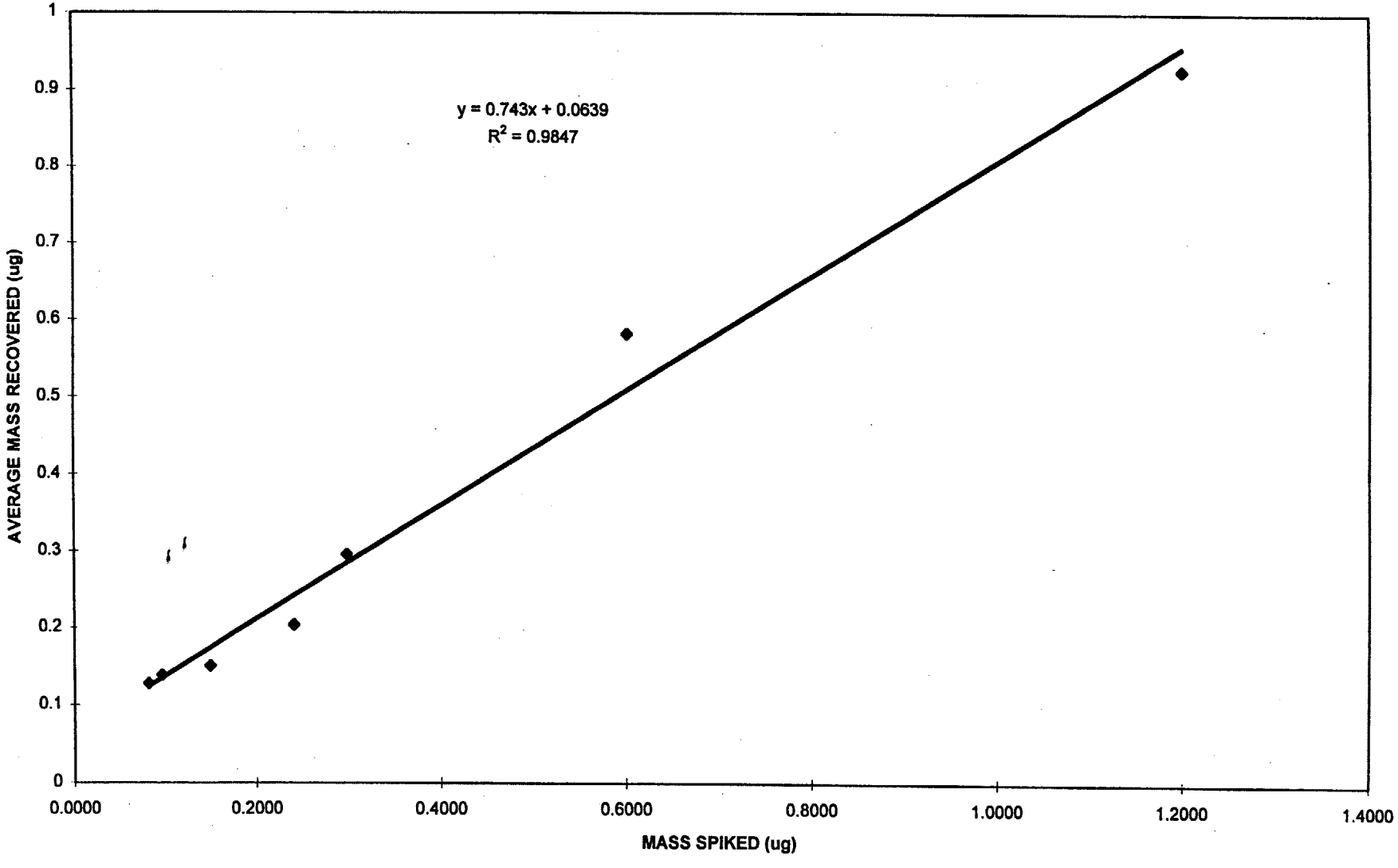
STUDY # 6329-135 SERUM

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
spike 124-3	0.205	0.1	2.0	0.004	124	138%	4.10	0.298	0.410
spike 124-4	0.0799	0.1	2.0	0.004	124	54%	1.60	0.298	0.160
SPIKE100-1	0.0659	0.1	2.0	0.004	100	55%	1.32	0.240	0.132
SPIKE100-2	0.0855	0.1	2.0	0.004	100	71%	1.71	0.240	0.171
SPIKE100-3	0.0851	0.1	2.0	0.004	100	71%	1.70	0.240	0.170
BLANK	0.0454	0.1	2.0				0.908		0.0908
BLANK	0.0222	0.1	2.0				0.444		0.0444
F52978 DAY22	0.0225	0.1	2.0				0.449		0.0449
F52980 DAY22	0.0258	0.1	2.0				0.516		0.0516
F52991 DAY22	0.0290	0.1	2.0				0.581		0.0581
F52987 DAY22	0.0168	0.1	2.0				0.335		0.0335
F52988 DAY22	0.0125	0.1	2.0				0.249		0.0249
F52995 DAY22	0.0138	0.1	2.0				0.275		0.0275
SPIKE 40-1	0.0337	0.1	2.0	0.004	40	70%	0.673	0.096	0.0673
SPIKE 100-1	0.0850	0.1	2.0	0.004	100	71%	1.70	0.240	0.170

NORMAN SERUM CURVE 1

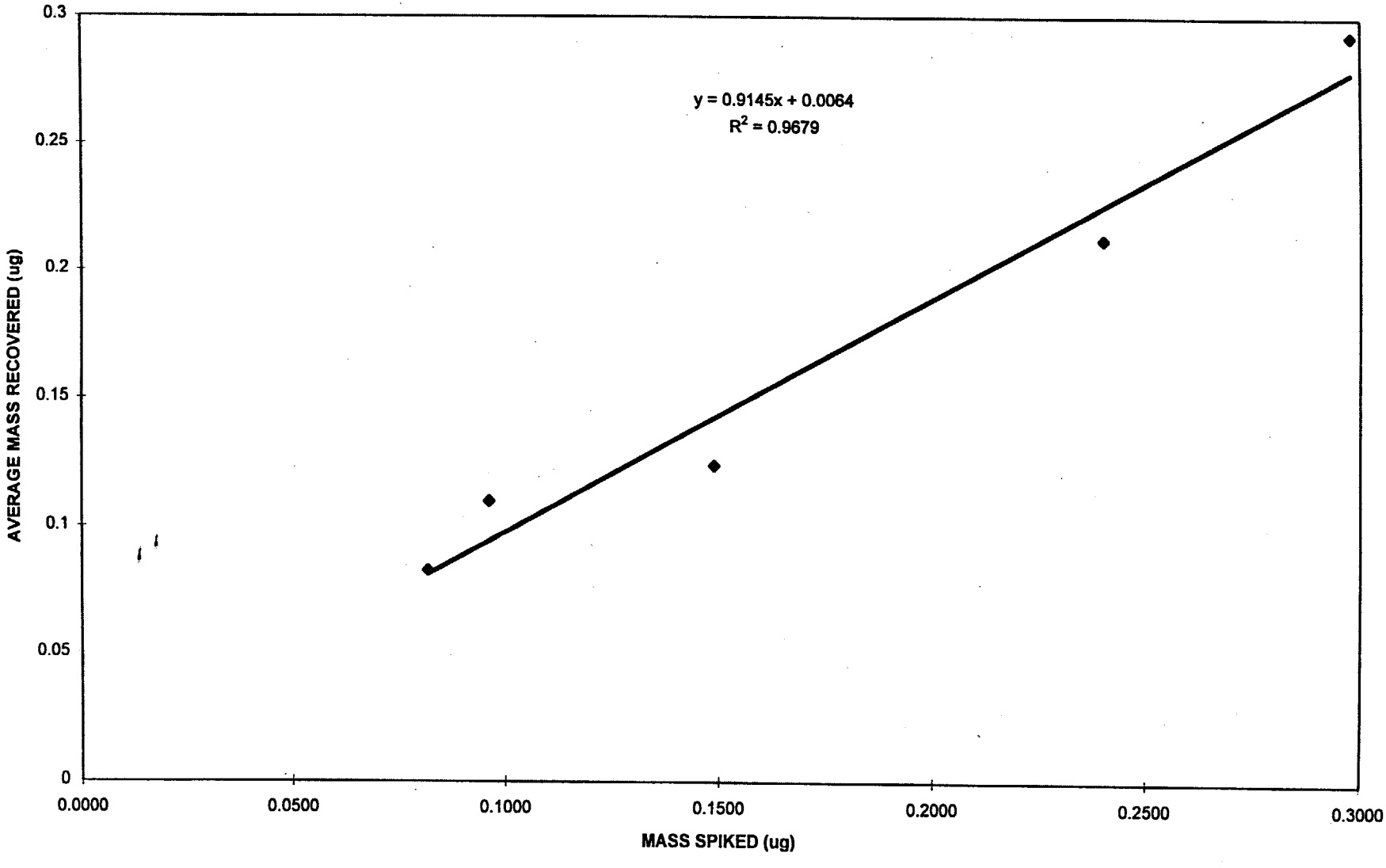
Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
4-ppm-1	0.07175	0.1	2.0	0.004	34	176%	1.4350	0.0817	0.1435	STDEV (34ppm):	0.0156
4-ppm-2	0.05614	0.1	2.0	0.004	34	138%	1.1228	0.0817	0.11228	%RSD:	12
4-ppm-3	0.06462	0.1	2.0	0.004	34	158%	1.2924	0.0817	0.12924	AVERAGE:	0.128
0-ppm-1	0.08668	0.1	2.0	0.004	40	180%	1.7336	0.0961	0.17336	STDEV (40ppm):	0.0241
0-ppm-2	0.06728	0.1	2.0	0.004	40	140%	1.3456	0.0961	0.13456	%RSD:	17
0-ppm-3	0.05939	0.1	2.0	0.004	40	124%	1.1878	0.0961	0.11878	AVERAGE:	0.139
0-ppm-4	0.06385	0.1	2.0	0.004	40	133%	1.2770	0.0961	0.1277		
2-ppm-1	0.07291	0.1	2.0	0.004	62	98%	1.4582	0.1489	0.14582	STDEV(62ppm):	0.00549
2-ppm-2	0.0753	0.1	2.0	0.004	62	101%	1.5060	0.1489	0.1506	%RSD:	3.6
2-ppm-3	0.07839	0.1	2.0	0.004	62	105%	1.5678	0.1489	0.15678	AVERAGE:	0.151
00-ppm-1	0.0902	0.1	2.0	0.004	100	75%	1.8040	0.2402	0.1804	STDEV (100ppm):	0.0224
00-ppm-2	0.1026	0.1	2.0	0.004	100	85%	2.0520	0.2402	0.2052	%RSD:	11
00-ppm-3	0.1126	0.1	2.0	0.004	100	94%	2.2520	0.2402	0.2252	AVERAGE:	0.204
24-ppm-1	0.1371	0.1	2.0	0.004	124	92%	2.7420	0.2978	0.2742	STDEV (124ppm):	0.0251
24-ppm-2	0.1451	0.1	2.0	0.004	124	97%	2.9020	0.2978	0.2902	%RSD:	8.5
24-ppm-3	0.1617	0.1	2.0	0.004	124	109%	3.2340	0.2978	0.3234	AVERAGE:	0.296
50-ppm-1	0.3217	0.1	2.0	0.004	250	107%	6.4340	0.6004	0.6434	STDEV (250ppm):	0.0821
50-ppm-2	0.2447	0.1	2.0	0.004	250	82%	4.8940	0.6004	0.4894	%RSD:	14
50-ppm-3	0.3078	0.1	2.0	0.004	250	103%	6.1560	0.6004	0.6156	AVERAGE:	0.583
00-ppm-1	0.4438	0.1	2.0	0.004	500	74%	8.8760	1.2008	0.8876	STDEV (500ppm):	0.0459
00-ppm-2	0.4584	0.1	2.0	0.004	500	76%	9.1680	1.2008	0.9168	%RSD:	5.0
00-ppm-3	0.4888	0.1	2.0	0.004	500	81%	9.7760	1.2008	0.9776	AVERAGE:	0.927

SERUM CURVE 1 NORMAN (07/25/95)



Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
SPIKE 34-1	0.0330	0.1	2.0	0.004	34	81%	0.660	0.0817	0.0660	STDEV (34ppm):	0.0152
SPIKE 34-2	0.0479	0.1	2.0	0.004	34	117%	0.957	0.0817	0.0957	%RSD:	18
SPIKE 34-3	0.0430	0.1	2.0	0.004	34	105%	0.861	0.0817	0.0861	AVERAGE:	0.0826
SPIKE 40-1	0.0682	0.1	2.0	0.004	40	142%	1.36	0.0961	0.136	STDEV (40ppm):	0.0234
SPIKE 40-2	0.0459	0.1	2.0	0.004	40	95%	0.917	0.0961	0.0917	%RSD:	21
SPIKE 40-3	0.0508	0.1	2.0	0.004	40	106%	1.02	0.0961	0.102	AVERAGE:	0.110
SPIKE 62PPM-1	0.0422	0.1	2.0	0.004	62	57%	0.845	0.149	0.0845	STDEV (62ppm):	0.0472
SPIKE 62PPM-2	0.0441	0.1	2.0	0.004	62	59%	0.882	0.149	0.0882	%RSD:	38
SPIKE 62PPM-3	0.0690	0.1	2.0	0.004	62	93%	1.38	0.149	0.138	AVERAGE:	0.124
SPIKE 62PPM-4	0.0922	0.1	2.0	0.004	62	124%	1.84	0.149	0.184		
SPIKE 100PPM-1	0.0962	0.1	2.0	0.004	100	80%	1.92	0.240	0.192	STDEV (100ppm):	0.0560
SPIKE 100PPM-2	0.159	0.1	2.0	0.004	100	132%	3.17	0.240	0.317	%RSD:	26
SPIKE 100PPM-3	0.0774	0.1	2.0	0.004	100	64%	1.55	0.240	0.155	AVERAGE:	0.213
SPIKE 100PPM-4	0.113	0.1	2.0	0.004	100	94%	2.25	0.240	0.225		
SPIKE 100PPM-5	0.0930	0.1	2.0	0.004	100	77%	1.86	0.240	0.186		
SPIKE 100PPM-6	0.100	0.1	2.0	0.004	100	83%	2.00	0.240	0.200		
SPIKE 124PPM-1	0.149	0.1	2.0	0.004	124	100%	2.97	0.298	0.297	STDEV (124ppm):	0.0108
SPIKE 124PPM-2	0.150	0.1	2.0	0.004	124	101%	3.00	0.298	0.300	%RSD:	3.7
SPIKE 124PPM-3	0.140	0.1	2.0	0.004	124	94%	2.80	0.298	0.280	AVERAGE:	0.293

SERUM CURVE 2 NORMAN (08/15/95)



9.11.4 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

DDW 12694
Skalar Data

RE: 6329-135 SERUM SAMPLES
AMDT 20795.1
Date of Analysis: 8/16, 8/21, and 8/22/95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using 0.10 mL of the serum. The gas is collected in 2.0 mL of 1:1 TISAB/Milli-Q water. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Debra D Wright

DNW 7/26/95
AMDT 20795.1
HWI 6329-135
S Kalar DATA

SUMMARY OF 6329-135
SERUM SAMPLES
AMDT 020795.1

	Sample ID	Fluoride in Sample (ppm) Day 1	Fluoride in Sample (ppm) Day 8	Fluoride in Sample (ppm) Day 15	Fluoride in Sample (ppm) Day22
	F52972	1.04	0.51	0.88	0.05
	F52973	0.98	ND	1.36	0.05
GROUP 1	F52979	1.02	0.78	1.00	0.04
Dose Level : 0	F52975	0.88	0.69	1.09	0.03
	F52976	0.84	0.52	0.82	0.03
	F52983	0.96	0.46	0.91	0.05
	F52986	1.78	0.54	0.25	0.04
	F52990	0.75	0.46	0.33	0.03
GROUP 2	F52997	0.47	0.61	0.23	0.04
Dose Level : 2 mg/kg	F52982	0.49	0.50	0.12	0.03
	F52994	0.56	1.85	0.33	0.04
	F53410	0.46	1.06	0.27	0.05
	F52984	0.81	1.05	0.14	0.05
	F52992	0.71	0.96	0.17	0.05
GROUP 3	F52996	0.62	1.29	0.18	0.05
Dose Level : 200 mg/kg	F52977	0.73	1.65	0.22	0.05
	F52989	0.71	1.26	2.07	0.04
	F52993	0.70	0.98	1.12	0.04
	F52978	0.81	0.82	1.17	0.03
	F52980	1.25	0.90	1.12	0.04
GROUP 4	F52991	1.31	1.22	1.06	0.03
Dose Level : 1000 mg/kg	F52987	1.03	1.09	0.94	0.02
	F52988	1.02	0.84	0.95	0.02
	F52995	0.92	0.86	0.77	0.03

1995-08-16 14:08 OutPut of : 950816A1
Operator : DDW
Date of the Analysis : 1995-08-16 08:58
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950816A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DL:TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.47	98%								
2	Drift	1.50	1.49	99%								
3	Wash		ND									
4	Standard 1	0.015	0.016	109%								
5	Standard 2	0.03	0.03	94%								
6	Standard 3	0.06	0.06	100%								
7	Standard 4	0.09	0.09	99%								
8	Standard 5	0.12	0.12	102%								
9	Standard 6	0.15	0.15	99%								
10	Standard 7	0.30	0.29	95%								
11	Standard 8	0.60	0.61	102%								
12	Standard 9	1.20	1.22	101%								
13	Standard 10	1.50	1.48	99%								
14	Drift	1.50	1.49	99%								
15	Wash		ND									
16	SPK 62-1		0.13		2.0	0.10	2.59	0.004	62.00	0.15	0.26	174%
17	SPK 250-1		0.35		2.0	0.10	6.92	0.004	250.0	0.60	0.69	115%
18	SPK 250-2		0.32		2.0	0.10	6.44	0.004	250.0	0.60	0.64	107%
19	BLANK		0.12		2.0	0.10	2.41					
20	BLANK		0.07		2.0	0.10	1.37					
21	BLANK		0.04		2.0	0.10	0.82					
22	F52986-1		0.09		2.0	0.10	1.78					
23	F52990-1		0.04		2.0	0.10	0.75					
24	F52997-1		0.02		2.0	0.10	0.47					
25	F52982-1		0.02		2.0	0.10	0.49					
26	Drift	1.50	1.48	98%								
27	Wash		ND									
28	F52994-1		0.03		2.0	0.10	0.56					
29	F53410-1		0.02		2.0	0.10	0.46					

000748

Ooo 9/26/95
AMDT 20795.1
#WI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	SPK 62-1		0.10		2.0	0.10	1.96	0.004	62.00	0.15	0.20	131%
31	SPK 250-1		0.29		2.0	0.10	5.88	0.004	250.0	0.60	0.59	98%
32	BLANK SERUM		0.06		2.0	0.10	1.25					
33	BLANK SERUM		0.05		2.0	0.10	1.04					
34	BLANK SERUM		0.06		2.0	0.10	1.13					
35	BLANK SERUM		0.05		2.0	0.10	0.97					
36	SPK 62-1		0.14		2.0	0.10	2.80	0.004	62.00	0.15	0.28	188%
37	SPK 62-2		0.11		2.0	0.10	2.29	0.004	62.00	0.15	0.23	154%
38	Drift	1.50	1.49	99%								
39	Wash		ND									
40	SPK 62-3		0.12		2.0	0.10	2.39	0.004	62.00	0.15	0.24	160%
41	SPK 250-1		0.25		2.0	0.10	5.08	0.004	250.0	0.60	0.51	85%
42	SPK 250-2		0.27		2.0	0.10	5.42	0.004	250.0	0.60	0.54	90%
43	F52984-1		0.04		2.0	0.10	0.81					
44	F52992-1		0.04		2.0	0.10	0.71					
45	F52996-1		0.03		2.0	0.10	0.62					
46	F52997-1		0.04		2.0	0.10	0.73					
47	F52984-1		0.04		2.0	0.10	0.71					
48	F52993-1		0.03		2.0	0.10	0.70					
49	F52978-1		0.04		2.0	0.10	0.81					
50	Drift	1.50	1.49	99%								
51	Wash		ND									
52	F52980-1		0.06		2.0	0.10	1.25					
53	F52991-1		0.07		2.0	0.10	1.31					
54	F52987-1		0.05		2.0	0.10	1.03					
55	SPK 62-1		0.11		2.0	0.10	2.10	0.004	62.00	0.15	0.21	141%
56	SPK 250-1		0.26		2.0	0.10	5.14	0.004	250.0	0.60	0.51	86%
57	Drift	1.50	1.50	100%								
58	Wash		ND									

1995-08-21 12:30 OutPut of : 950821A1
Operator : DDW
Date of the Analysis : 1995-08-21 07:55
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.47	98%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.014	96%								
5	Standard 2	0.03	0.03	99%								
6	Standard 3	0.06	0.06	104%								
7	Standard 4	0.09	0.09	99%								
8	Standard 5	0.12	0.12	99%								
9	Standard 6	0.15	0.15	101%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.22	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.54	103%								
15	Wash		ND									
16	SERUM BLK 1		0.08		2.0	0.10	1.53					
17	SERUM BLK 2		0.07		2.0	0.10	1.34					
18	SERUM BLK 3		0.06		2.0	0.10	1.12					
19	F52988-1		0.05		2.0	0.10	1.02					
20	F52995-1		0.05		2.0	0.10	0.92					
21	F52972-1		0.05		2.0	0.10	1.04					
22	F52973-1		0.05		2.0	0.10	0.98					
23	F52979-1		0.05		2.0	0.10	1.02					
24	F52975-1		0.04		2.0	0.10	0.88					
25	F52976-1		0.04		2.0	0.10	0.84					
26	Drift	1.50	1.53	102%								
27	Wash		ND									
28	F52983-1		0.05		2.0	0.10	0.96					
29	SPK 40-1		0.07		2.0	0.10	1.34	0.004	40.00	0.10	0.13	140%

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	SPK 100-1		0.14		2.0	0.10	2.83	0.004	100.0	0.24	0.28	118%
31	BLK-1		0.03		2.0	0.10	0.69					
32	BLK-2		0.03		2.0	0.10	0.64					
33	BLK-3		0.02		2.0	0.10	0.49					
34	SPK 40-1		0.06		2.0	0.10	1.29	0.004	40.00	0.10	0.13	134%
35	SPK 40-2		0.06		2.0	0.10	1.23	0.004	40.00	0.10	0.12	128%
36	SPK 100-1		0.13		2.0	0.10	2.50	0.004	100.0	0.24	0.25	104%
37	SPK 100-2		0.12		2.0	0.10	2.44	0.004	100.0	0.24	0.24	102%
38	Drift	1.50	1.54	103%								
39	Wash		ND									
40	SPK 100-3		0.14		2.0	0.10	2.70	0.004	100.0	0.24	0.27	113%
41	BLK		0.03		2.0	0.10	0.69					
42	F52972-8		0.03		2.0	0.10	0.51					
43	F52973-8		ND		2.0	0.10	ND					
44	F52979-8		0.04		2.0	0.10	0.78					
45	F52975-8		0.03		2.0	0.10	0.69					
46	F52976-8		0.03		2.0	0.10	0.52					
47	F52983-8		0.02		2.0	0.10	0.46					
48	F52986-8		0.03		2.0	0.10	0.54					
49	F52990-8		0.02		2.0	0.10	0.46					
50	Drift	1.50	1.53	102%								
51	Wash		ND									
52	F52997-8		0.03		2.0	0.10	0.61					
53	F52982-8		0.02		2.0	0.10	0.50					
54	SPK 62-1		0.05		2.0	0.10	1.10	0.004	62.00	0.15	0.11	74%
55	SPK 62-2		0.11		2.0	0.10	2.27	0.004	62.00	0.15	0.23	152%
56	SPK 124-1		0.14		2.0	0.10	2.75	0.004	124.0	0.30	0.27	92%
57	SPK 124-2		0.17		2.0	0.10	3.42	0.004	124.0	0.30	0.34	115%
58	BLK		0.10		2.0	0.10	2.07					
59	F52994-8		0.09		2.0	0.10	1.85					
60	F53410-8		0.05		2.0	0.10	1.06					
61	F52984-8		0.05		2.0	0.10	1.05					
62	Drift	1.50	1.55	103%								
63	Wash		ND									
64	F52992-8		0.05		2.0	0.10	0.96					
65	F52996-8		0.06		2.0	0.10	1.29					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
66	F52997-8		0.08		2.0	0.10	1.65					
67	F52989-8		0.06		2.0	0.10	1.26					
68	F52993-8		0.05		2.0	0.10	0.98					
69	F52978-8		0.04		2.0	0.10	0.82					
70	F52980-8		0.05		2.0	0.10	0.90					
71	SPK 40-1		0.08		2.0	0.10	1.51	0.004	40.00	0.10	0.15	157%
72	SPK 124-1		0.17		2.0	0.10	3.32	0.004	124.0	0.30	0.33	111%
73	BLK		0.09		2.0	0.10	1.79					
74	Drift	1.50	1.54	102%								
75	Wash		ND									
76	F52991-8		0.06		2.0	0.10	1.22					
77	F52987-8		0.05		2.0	0.10	1.09					
78	F52988-8		0.04		2.0	0.10	0.84					
79	F52995-8		0.04		2.0	0.10	0.86					
80	F52972-15		0.04		2.0	0.10	0.88					
81	F52973-15		0.07		2.0	0.10	1.36					
82	F52979-15		0.05		2.0	0.10	1.00					
83	F52975-15		0.05		2.0	0.10	1.09					
84	F52976-15		0.04		2.0	0.10	0.82					
85	F52983-15		0.05		2.0	0.10	0.91					
86	Drift	1.50	1.57	105%								
87	Wash		ND									
88	SPK 40-1		0.08		2.0	0.10	1.52	0.004	40.00	0.10	0.15	158%
89	SPK 124-1		0.17		2.0	0.10	3.30	0.004	124.0	0.30	0.33	111%
90	Drift	1.50	1.53	102%								
91	Wash		ND									

1995-08-21 16:58 OutPut of : 950821B1
Operator : DDW
Date of the Analysis : 1995-08-21 12:29
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821B1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.49	99%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.016	105%								
5	Standard 2	0.03	0.03	95%								
6	Standard 3	0.06	0.06	102%								
7	Standard 4	0.09	0.09	101%								
8	Standard 5	0.12	0.12	98%								
9	Standard 6	0.15	0.15	100%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.22	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.44	96%								
15	Wash		ND									
16	SERUM BLK 1		0.03		2.0	0.10	0.63					
17	SERUM BLK 2		0.02		2.0	0.10	0.37					
18	SPK 40-1		0.04		2.0	0.10	0.81	0.004	40.00	0.10	0.08	85%
19	SPK 40-2		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
20	SPK 40-3		0.05		2.0	0.10	1.03	0.004	40.00	0.10	0.10	107%
21	SPK 124-1		0.14		2.0	0.10	2.76	0.004	124.0	0.30	0.28	93%
22	SPK 124-2		0.14		2.0	0.10	2.88	0.004	124.0	0.30	0.29	97%
23	SPK 124-3		0.14		2.0	0.10	2.77	0.004	124.0	0.30	0.28	93%
24	BLK		0.03		2.0	0.10	0.65					
25	F52986-15		0.01		2.0	0.10	0.25					
26	Drift	1.50	1.41	94%								
27	Wash		ND									
28	F52990-15		0.02		2.0	0.10	0.33					
29	F52997-15		0.01		2.0	0.10	0.23					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	F52982-15		0.01		2.0	0.10	0.12					
31	F52994-15		0.02		2.0	0.10	0.33					
32	F53410-15		0.01		2.0	0.10	0.27					
33	F52984-15		0.01		2.0	0.10	0.14					
34	F52992-15		0.01		2.0	0.10	0.17					
35	F52996-15		0.01		2.0	0.10	0.18					
36	F52977-15		0.01		2.0	0.10	0.22					
37	SPK 40-1		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
38	Drift	1.50	1.43	96%								
39	Wash		ND									
40	SPK 124-1		0.19		2.0	0.10	3.82					
41	BLK		0.07		2.0	0.10	1.43					
42	F52989-15		0.10		2.0	0.10	2.07					
43	F52993-15		0.06		2.0	0.10	1.12					
44	F52978-15		0.06		2.0	0.10	1.17					
45	F52980-15		0.06		2.0	0.10	1.12					
46	F52991-15		0.05		2.0	0.10	1.06					
47	F52987-15		0.05		2.0	0.10	0.94					
48	F52988-15		0.05		2.0	0.10	0.95					
49	F52995-15		0.04		2.0	0.10	0.77					
50	Drift	1.50	1.45	97%								
51	Wash		ND									
52	F52972-22		0.05		2.0	0.10	0.91					
53	F52973-22		0.05		2.0	0.10	1.02					
54	SPK 40-1		0.10		2.0	0.10	2.03	0.004	40.00	0.10	0.20	212%
55	SPK 40-2		0.07		2.0	0.10	1.31	0.004	40.00	0.10	0.13	136%
56	SPK 124-1		0.14		2.0	0.10	2.78	0.004	124.0	0.30	0.28	93%
57	BLK		0.06		2.0	0.10	1.19					
58	F52979-22		0.04		2.0	0.10	0.82					
59	F52975-22		0.03		2.0	0.10	0.68					
60	F52976-22		0.03		2.0	0.10	0.65					
61	F52983-22		0.05		2.0	0.10	0.91					
62	Drift	1.50	1.42	95%								
63	Wash		ND									
64	F52986-22		0.04		2.0	0.10	0.88					
65	F52990-22		0.03		2.0	0.10	0.69					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
66	F52997-22		0.04		2.0	0.10	0.73					
67	F52982-22		0.03		2.0	0.10	0.57					
68	F52994-22		0.04		2.0	0.10	0.89					
69	F53410-22		0.05		2.0	0.10	0.92					
70	SPK 40-1		0.09		2.0	0.10	1.79	0.004	40.00	0.10	0.18	186%
71	SPK 124-1		0.17		2.0	0.10	3.36	0.004	124.0	0.30	0.34	113%
72	SPK 62-1		0.12		2.0	0.10	2.42	0.004	62.00	0.15	0.24	163%
73	BLK		0.06		2.0	0.10	1.24					
74	Drift	1.50	1.47	98%								
75	Wash		ND									
76	F52984-22		0.05		2.0	0.10	0.94					
77	F52992-22		0.05		2.0	0.10	0.91					
78	F52996-22		0.05		2.0	0.10	0.96					
79	F52977-22		0.05		2.0	0.10	0.93					
80	F52989-22		0.04		2.0	0.10	0.88					
81	F52993-22		0.04		2.0	0.10	0.79					
82	SPK 40-1		0.09		2.0	0.10	1.71	0.004	40.00	0.10	0.17	178%
83	SPK 124-1		0.15		2.0	0.10	2.92	0.004	124.0	0.30	0.29	98%
84	Drift	1.50	1.46	97%								
85	Wash		ND									

1995-08-22 09:05 OutPut of : 950822A1
Operator : DDW
Date of the Analysis : 1995-08-22 06:49
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950822A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.46	97%								
2	Drift	1.50	1.48	98%								
3	Wash		ND									
4	Standard 1	0.015	0.016	104%								
5	Standard 2	0.03	0.03	94%								
6	Standard 3	0.06	0.06	104%								
7	Standard 4	0.09	0.09	101%								
8	Standard 5	0.12	0.12	97%								
9	Standard 6	0.15	0.15	101%								
10	Standard 7	0.30	0.28	92%								
11	Standard 8	0.60	0.62	104%								
12	Standard 9	1.20	1.23	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.54	102%								
15	Wash		ND									
16	SERUM BLK 1		0.05		2.0	0.10	0.94					
17	SERUM BLK 2		0.03		2.0	0.10	0.67					
18	SPK 40-1		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
19	SPK 40-2		0.06		2.0	0.10	1.14	0.004	40.00	0.10	0.11	119%
20	SPK 40-3		0.06		2.0	0.10	1.15	0.004	40.00	0.10	0.12	120%
21	SPK 40-4		0.06		2.0	0.10	1.22	0.004	40.00	0.10	0.12	127%
22	SPK 124-1		0.08		2.0	0.10	1.66	0.004	124.0	0.30	0.17	56%
23	SPK 124-2		0.13		2.0	0.10	2.57	0.004	124.0	0.30	0.26	86%
24	SPK 124-3		0.28		2.0	0.10	5.56	0.004	124.0	0.30	0.56	187%
25	SPK 124-4		0.12		2.0	0.10	2.34	0.004	124.0	0.30	0.23	79%
26	Drift	1.50	1.53	102%								
27	Wash		ND									
28	SPK 100-1		0.11		2.0	0.10	2.12	0.004	100.0	0.24	0.21	88%
29	SPK 100-2		0.13		2.0	0.10	2.63	0.004	100.0	0.24	0.26	110%
30	SPK 100-3		0.13		2.0	0.10	2.56	0.004	100.0	0.24	0.26	106%

000756

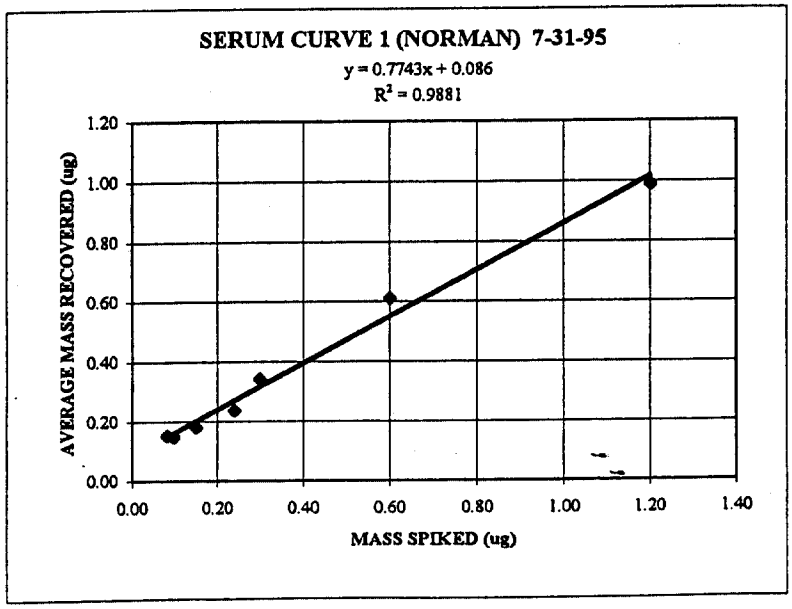
WDW '126.82
AMDT 20795.1
HWI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
31	BLK		0.08		2.0	0.10	1.51					
32	BLK		0.04		2.0	0.10	0.78					
33	F52978-22		0.03		2.0	0.10	0.67					
34	F52980-22		0.04		2.0	0.10	0.85					
35	F52991-22		0.03		2.0	0.10	0.58					
36	F52987-22		0.02		2.0	0.10	0.50					
37	F52988-22		0.02		2.0	0.10	0.34					
38	Drift	1.50	1.53	102%								
39	Wash		ND									
40	F52995-22		0.03		2.0	0.10	0.54					
41	BLANK TISAB		ND									
42	SPK 100-1		0.11		2.0	0.10	2.12	0.004	100.0	0.24	0.21	88%
43	Drift	1.50	1.55	103%								
44	Wash		ND									

UDW '120143
AMDT 20795.1
HWI 6329-135
Skalar Data

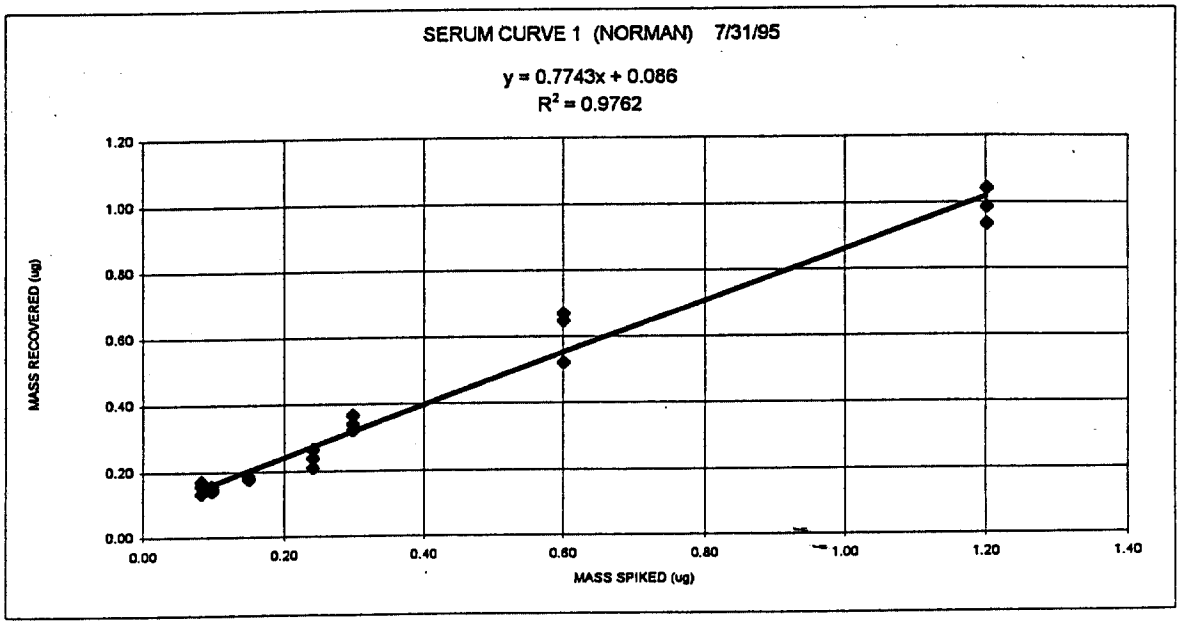
SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Average Mass Recovered (ug F-)	% Recovery
Spk 34-1	0.09	2.0	0.004	34.00			
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.15	188%
Spk 34-3	0.08	2.0	0.004	34.00			
Spk 40-1	0.08	2.0	0.004	40.00			
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.15	155%
Spk 40-3	0.07	2.0	0.004	40.00			
Spk 62-1	0.09	2.0	0.004	62.00			
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	121%
Spk 62-3	0.09	2.0	0.004	62.00			
Spk 100-1	0.11	2.0	0.004	100.0			
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	99%
Spk 100-3	0.13	2.0	0.004	100.0			
Spk 124-1	0.16	2.0	0.004	124.0			
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	115%
Spk 124-3	0.18	2.0	0.004	124.0			
Spk 250-1	0.33	2.0	0.004	250.0			
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.61	102%
Spk 250-3	0.32	2.0	0.004	250.0			
Spk 500-1	0.47	2.0	0.004	500.0			
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%
Spk 500-3	0.52	2.0	0.004	500.0			



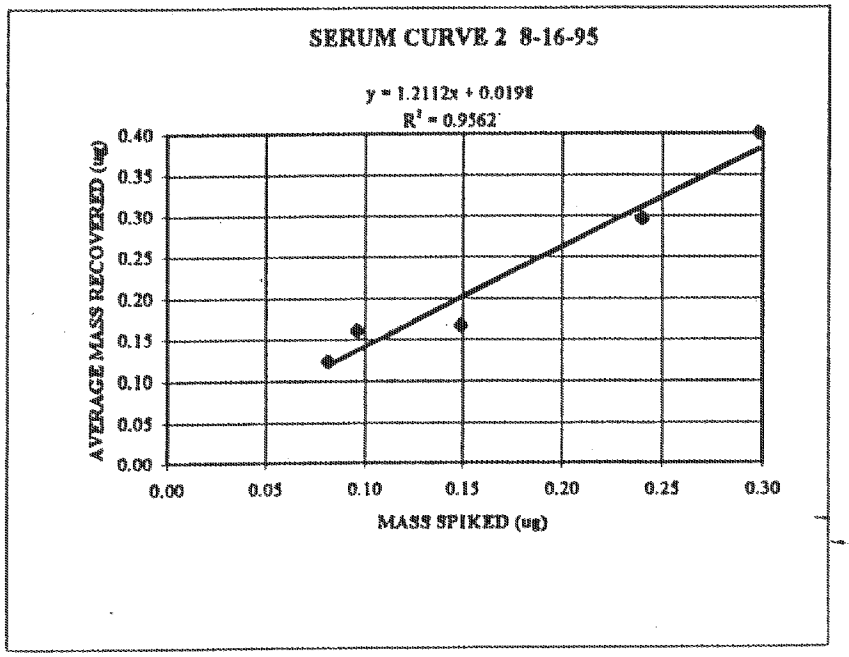
SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery		
Spk 34-1	0.09	2.0	0.004	34.00	0.08	0.17	211%	STANDARD DEVIATION :	0.2450
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.13	163%	% RSD :	12.9998
Spk 34-3	0.08	2.0	0.004	34.00	0.08	0.16	191%		
Spk 40-1	0.08	2.0	0.004	40.00	0.10	0.16	164%	STANDARD DEVIATION :	0.0826
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.14	147%	% RSD :	5.3307
Spk 40-3	0.07	2.0	0.004	40.00	0.10	0.15	154%		
Spk 62-1	0.09	2.0	0.004	62.00	0.15	0.18	120%	STANDARD DEVIATION :	0.0263
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	119%	% RSD :	2.1670
Spk 62-3	0.09	2.0	0.004	62.00	0.15	0.18	124%		
Spk 100-1	0.11	2.0	0.004	100.0	0.24	0.21	88%	STANDARD DEVIATION :	0.1138
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	100%	% RSD :	11.4530
Spk 100-3	0.13	2.0	0.004	100.0	0.24	0.27	110%		
Spk 124-1	0.16	2.0	0.004	124.0	0.30	0.32	108%	STANDARD DEVIATION :	0.0778
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	114%	% RSD :	6.7516
Spk 124-3	0.18	2.0	0.004	124.0	0.30	0.37	124%		
Spk 250-1	0.33	2.0	0.004	250.0	0.60	0.67	111%	STANDARD DEVIATION :	0.1318
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.52	87%	% RSD :	12.9196
Spk 250-3	0.32	2.0	0.004	250.0	0.60	0.65	108%		
Spk 500-1	0.47	2.0	0.004	500.0	1.20	0.94	78%	STANDARD DEVIATION :	0.0442
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%	% RSD :	5.3672
Spk 500-3	0.52	2.0	0.004	500.0	1.20	1.04	87%		



SERUM CURVE 2
8-16-95
NORMAN

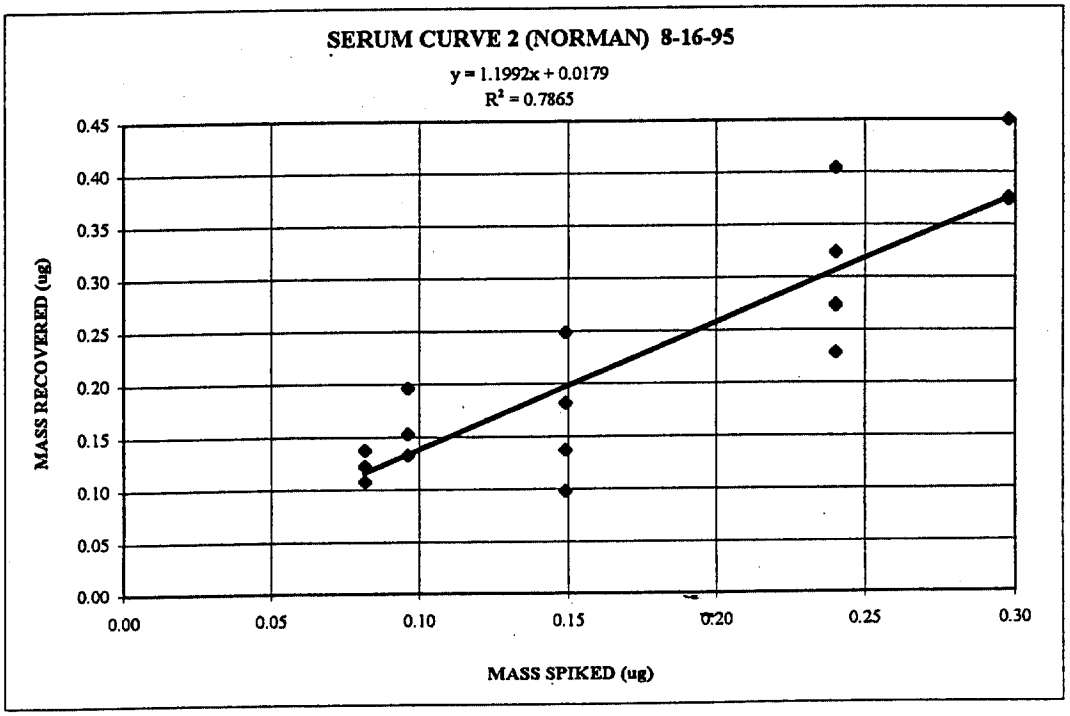
Sample ID	Sample Result (ppm)	DILUSAB (mL)	mL FC-95 Solution Spiked	Conc. FC-95 Solution (ppm)	Mass Spiked (ug FC)	Average Mass Recovered (ug FC)	% Accuracy
SPK 34-1	0.05	2.0	0.004	34.00			
SPK 34-2	0.07	2.0	0.004	34.00	0.08	0.12	150%
SPK 34-3	0.06	2.0	0.004	34.00			
SPK 40-1	0.10	2.0	0.004	40.00			
SPK 40-2	0.07	2.0	0.004	40.00	0.10	0.16	167%
SPK 40-3	0.08	2.0	0.004	40.00			
SPK 62-1	0.05	2.0	0.004	62.00			
SPK 62-2	0.07	2.0	0.004	62.00	0.15	0.17	112%
SPK 62-3	0.09	2.0	0.004	62.00			
SPK 62-4	0.12	2.0	0.004	62.00			
SPK 100-1	0.14	2.0	0.004	100.0			
SPK 100-2	0.20	2.0	0.004	100.0			
SPK 100-3	0.11	2.0	0.004	100.0	0.24	0.30	123%
SPK 100-4	0.16	2.0	0.004	100.0			
SPK 100-5	0.14	2.0	0.004	100.0			
SPK 100-6	0.14	2.0	0.004	100.0			
SPK 124-1	0.19	2.0	0.004	124.0			
SPK 124-2	0.23	2.0	0.004	124.0	0.30	0.40	134%
SPK 124-3	0.19	2.0	0.004	124.0			



Copy of Original
DAW
9/20/95

SERUM CURVE 2
8-16-95
NORMAN

Sample ID	Skalar Result (ppm)	DI H2SAB final vol (mL)	mL FC 95 Solution Spiked	Conc. FC 95 Soln (ppm)	Mass Spiked (ug Fe)	Mass Recovered (ug Fe)	% Recovery		
SPK 34-1	0.05	2.0	0.004	34.00	0.08	0.11	132%	STANDARD DEVIATION :	0.1837
SPK 34-2	0.07	2.0	0.004	34.00	0.08	0.14	169%	% RSD :	12.2150
SPK 34-3	0.06	2.0	0.004	34.00	0.08	0.12	150%		
SPK 40-1	0.10	2.0	0.004	40.00	0.10	0.20	204%	STANDARD DEVIATION :	0.3354
SPK 40-2	0.07	2.0	0.004	40.00	0.10	0.13	139%	% RSD :	20.0349
SPK 40-3	0.08	2.0	0.004	40.00	0.10	0.15	159%		
SPK 62-1	0.05	2.0	0.004	62.00	0.15	0.10	66%	STANDARD DEVIATION :	0.4333
SPK 62-2	0.07	2.0	0.004	62.00	0.15	0.14	92%	% RSD :	38.7472
SPK 62-3	0.09	2.0	0.004	62.00	0.15	0.18	122%		
SPK 62-4	0.12	2.0	0.004	62.00	0.15	0.25	167%		
SPK 100-1	0.14	2.0	0.004	100.0	0.24	0.27	114%	STANDARD DEVIATION :	0.2535
SPK 100-2	0.20	2.0	0.004	100.0	0.24	0.40	168%	% RSD :	20.5563
SPK 100-3	0.11	2.0	0.004	100.0	0.24	0.23	95%		
SPK 100-4	0.16	2.0	0.004	100.0	0.24	0.32	135%		
SPK 100-5	0.14	2.0	0.004	100.0	0.24	0.27	114%		
SPK 100-6	0.14	2.0	0.004	100.0	0.24	0.27	114%		
SPK 124-1	0.19	2.0	0.004	124.0	0.30	0.38	126%	STANDARD DEVIATION :	0.1454
SPK 124-2	0.23	2.0	0.004	124.0	0.30	0.45	151%	% RSD :	10.8282
SPK 124-3	0.19	2.0	0.004	124.0	0.30	0.37	126%		



DDW 8/25/95
AMDT 20795.1
HWI 6329-135
Serum Curve 1-11

1995-08-16 14:10 OutPut of : 950816A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-16 08:58

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950816A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00065
a0 = -1.15706

Fluoride L
Calibration order = 2

Correlation : r = 0.99717

Result = $a2 * x^2 + a1 * x + a0$

a2 = 0.00000
a1 = 0.00018
a0 = 0.00912

Sampler
Type : SA1000
Number : 1
Sample Time : 50 sec.
Wash Time : 120 sec.
Air Time : 1 sec.
Take up : Single
sPecial : None
needle Height : 70 mm.

Diluter
needle Height : 80 mm
dilution Factor : 10
dilution Volume : 2.5 ml.
Resample : 1
Dilution runs : 1

User file : . TXT
Reproces : No

1995-08-16 14:10 OutPut of : 950816A1

Fluoride 1.5

```
Path number    :  3
Signal type    : Debubbled
Decolor        : Yes
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off

s1   sTandard  : Ignore
s2   sTandard  : Ignore
s3   sTandard  : Ignore
s4   sTandard  : Ignore
s5   sTandard  : Ignore
s6   sTandard  :    0.150
s7   sTandard  :    0.300
s8   sTandard  :    0.600
s9   sTandard  :    1.200
s10  sTandard  :    1.500
Order : Inverse Logarithm
Dimension : PPM
start Value    : 500  DU
trigger Limit  : 1800 Sec
Peak shape     : Pointed
stArt ignore   : 60   Sec
eNd   ignore   : 120  Sec
Measure window : 75   %
Filter         : No
Regeneration   : No
formUla    :
output     : ##.###
```

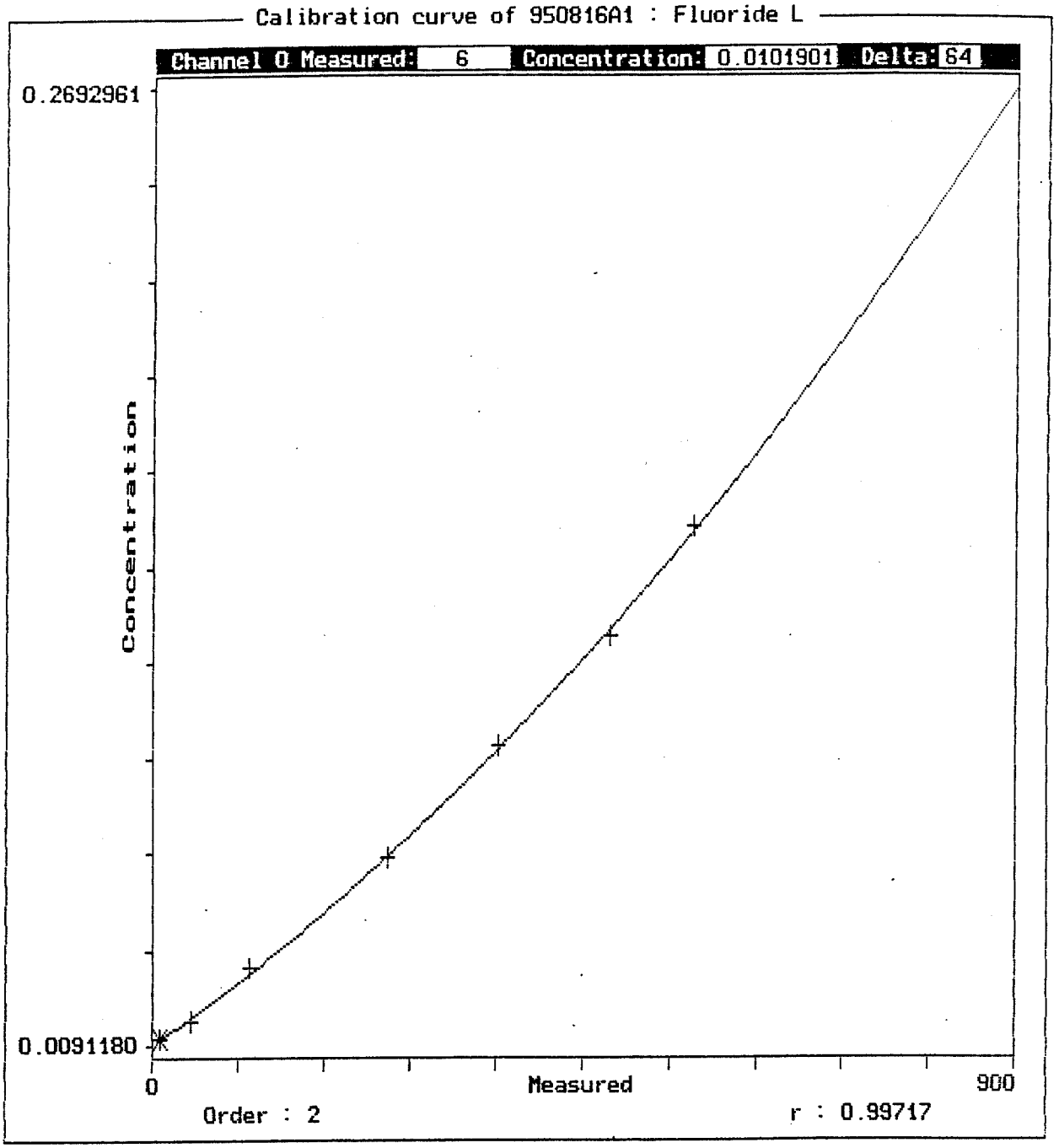
Fluoride L

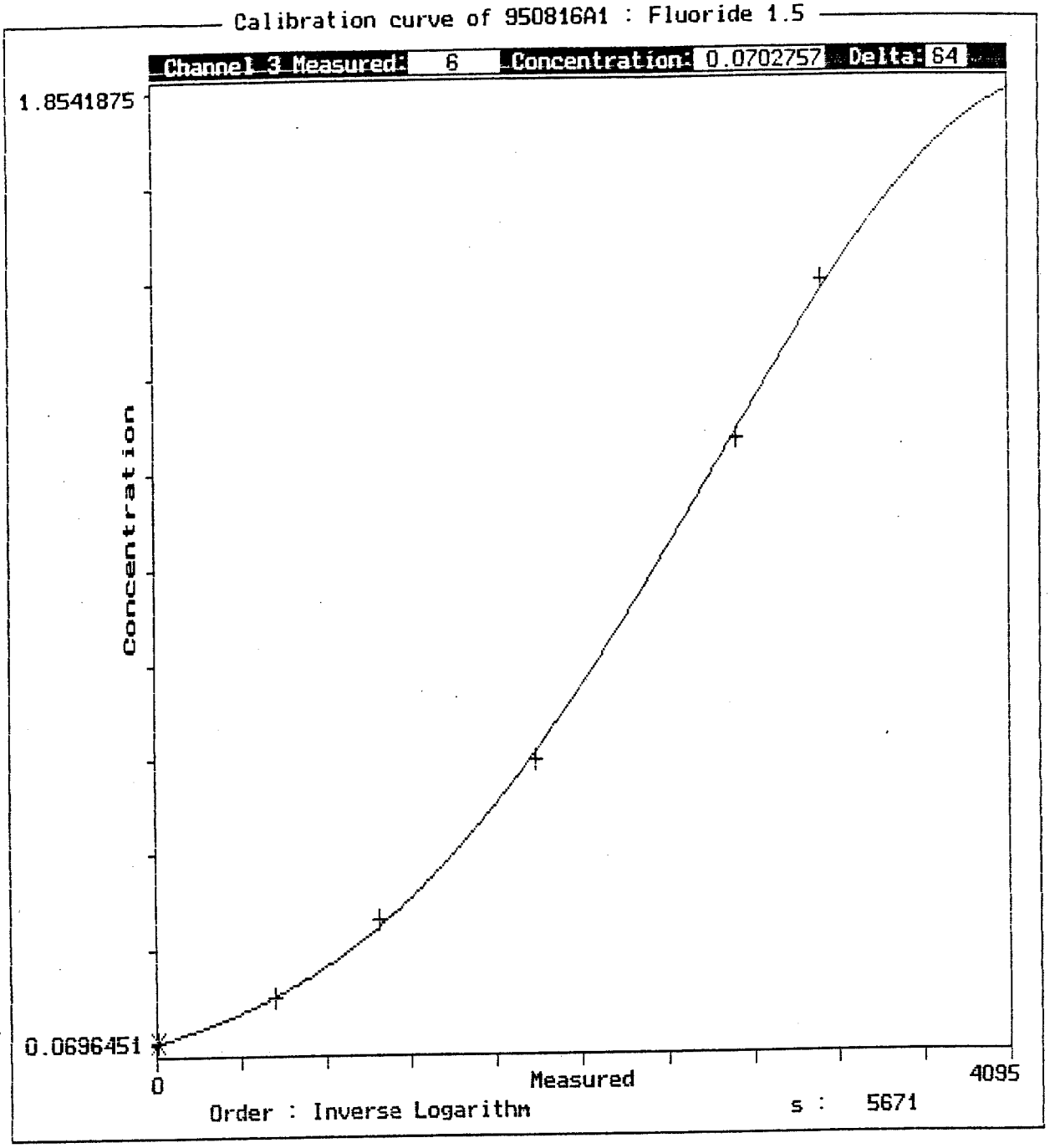
```
Path number    :  0
Signal type    : Debubbled
Decolor        : No
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off
```

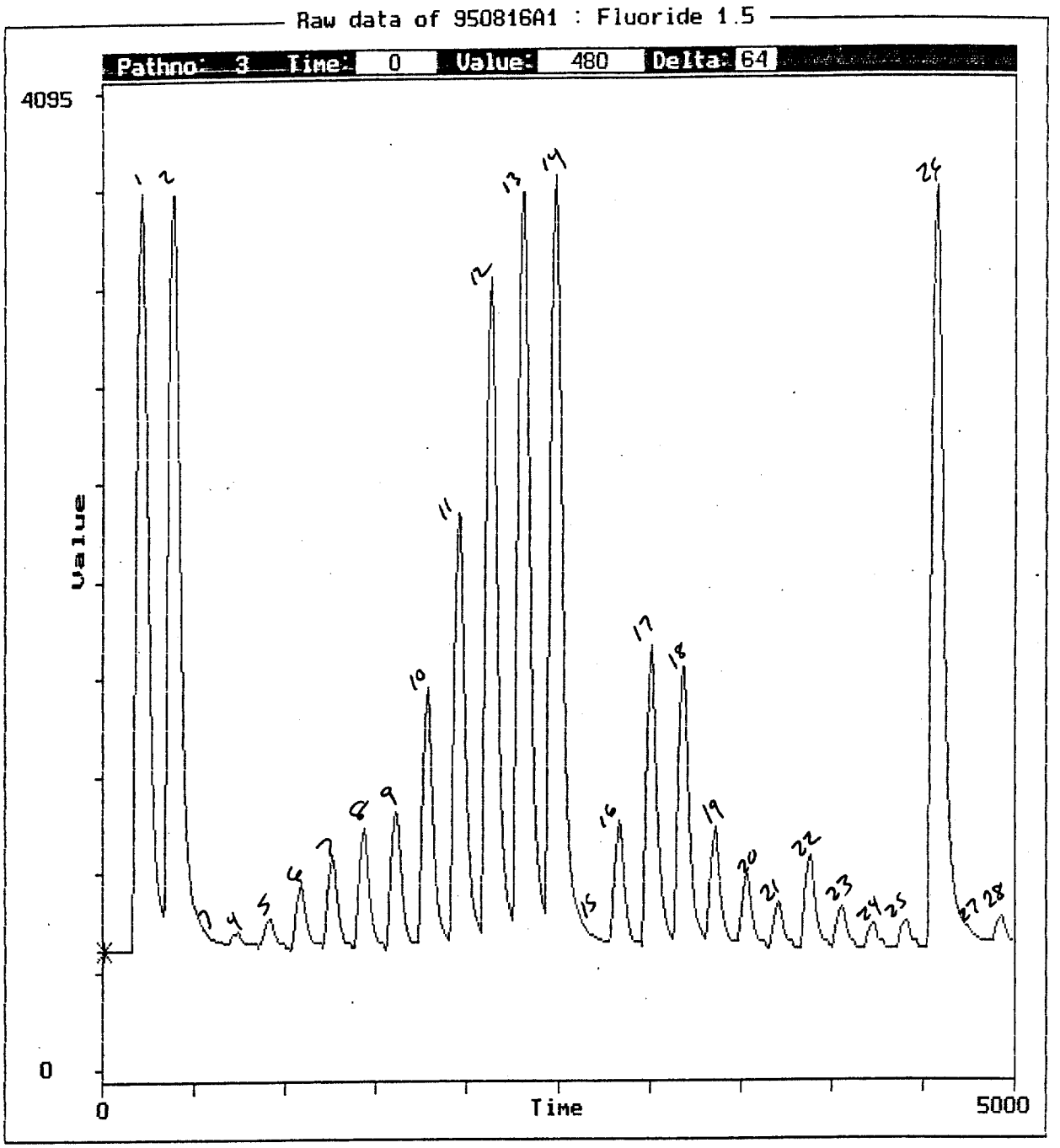
```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.070	65	4	0.0091	0
1	t	Tracer	3	1.472	207	4	1.8335	0
2	d	Drift	3	1.486	383	4	1.8558	0
3	w	Wash	3	0.070	619	4	0.0091	0
4	s1	Standard 1	3	0.074	729	4	0.0164	0
5	s2	Standard 2	3	0.081	907	4	0.0281	0
6	s3	Standard 3	3	0.100	1081	4	0.0601	0
7	s4	Standard 4	3	0.117	1258	4	0.0894	0
8	s5	Standard 5	3	0.137	1434	4	0.1221	0
9	s6	Standard 6	3	0.154	1608	4	0.1488	0
10	s7	Standard 7	3	0.285	1784	4	0.3400	0
11	s8	Standard 8	3	0.614	1958	4	0.7483	0
12	s9	Standard 9	3	1.217	2133	4	1.4687	0
13	s10	Standard 10	3	1.479	2308	4	1.8442	0
14	d	Drift	3	1.490	2484	4	1.8617	0
15	w	Wash	3	0.070	2726	4	0.0091	0
16	u	SPK 62-1	3	0.142	2830	4	0.1296	0
17	u	SPK 250-1	3	0.346	3011	4	0.4211	0
18	u	SPK 250-2	3	0.322	3187	4	0.3892	0
19	u	BLANK	3	0.136	3361	4	0.1206	0
20	u	BLANK	3	0.105	3536	4	0.0686	0
21	u	BLANK	3	0.088	3710	4	0.0410	0
22	u	F52986-1	3	0.117	3886	4	0.0889	0
23	u	F52990-1	3	0.086	4062	4	0.0377	0
24	u	F52997-1	3	0.078	4236	4	0.0234	0
25	u	F52982-1	3	0.079	4410	4	0.0247	0
26	d	Drift	3	1.477	4586	4	1.8413	0
27	w	Wash	3	0.070	4825	4	0.0091	0
28	u	F52994-1	3	0.081	4934	4	0.0281	0
29	u	F53410-1	3	0.078	5110	4	0.0228	0
30	u	SPK 62-1	3	0.122	5288	4	0.0978	0
31	u	SPK 250-1	3	0.294	5462	4	0.3525	0
32	u	BLANK SERUM	3	0.101	5635	4	0.0625	0
33	u	BLANK SERUM	3	0.095	5810	4	0.0519	0
34	u	BLANK SERUM	3	0.098	5986	4	0.0566	0
35	u	BLANK SERUM	3	0.093	6161	4	0.0485	0
36	u	SPK 62-1	3	0.148	6335	4	0.1400	0
37	u	SPK 62-2	3	0.132	6510	4	0.1145	0
38	d	Drift	3	1.485	6684	4	1.8539	0
39	w	Wash	3	0.070	6911	4	0.0091	0
40	u	SPK 62-3	3	0.136	7034	4	0.1194	0
41	u	SPK 250-1	3	0.254	7210	4	0.2971	0
42	u	SPK 250-2	3	0.271	7382	4	0.3208	0
43	u	F52984-1	3	0.088	7557	4	0.0407	0
44	u	F52992-1	3	0.085	7734	4	0.0356	0
45	u	F52996-1	3	0.082	7910	4	0.0310	0
46	u	F52997-1	3	0.086	8086	4	0.0364	0
47	u	F52984-1	3	0.085	8258	4	0.0356	0
48	u	F52993-1	3	0.085	8434	4	0.0348	0
49	u	F52978-1	3	0.088	8608	4	0.0407	0
50	d	Drift	3	1.488	8785	4	1.8587	0
51	w	Wash	3	0.070	9004	4	0.0091	0
52	u	F52980-1	3	0.101	9131	4	0.0625	0
53	u	F52991-1	3	0.103	9311	4	0.0654	0

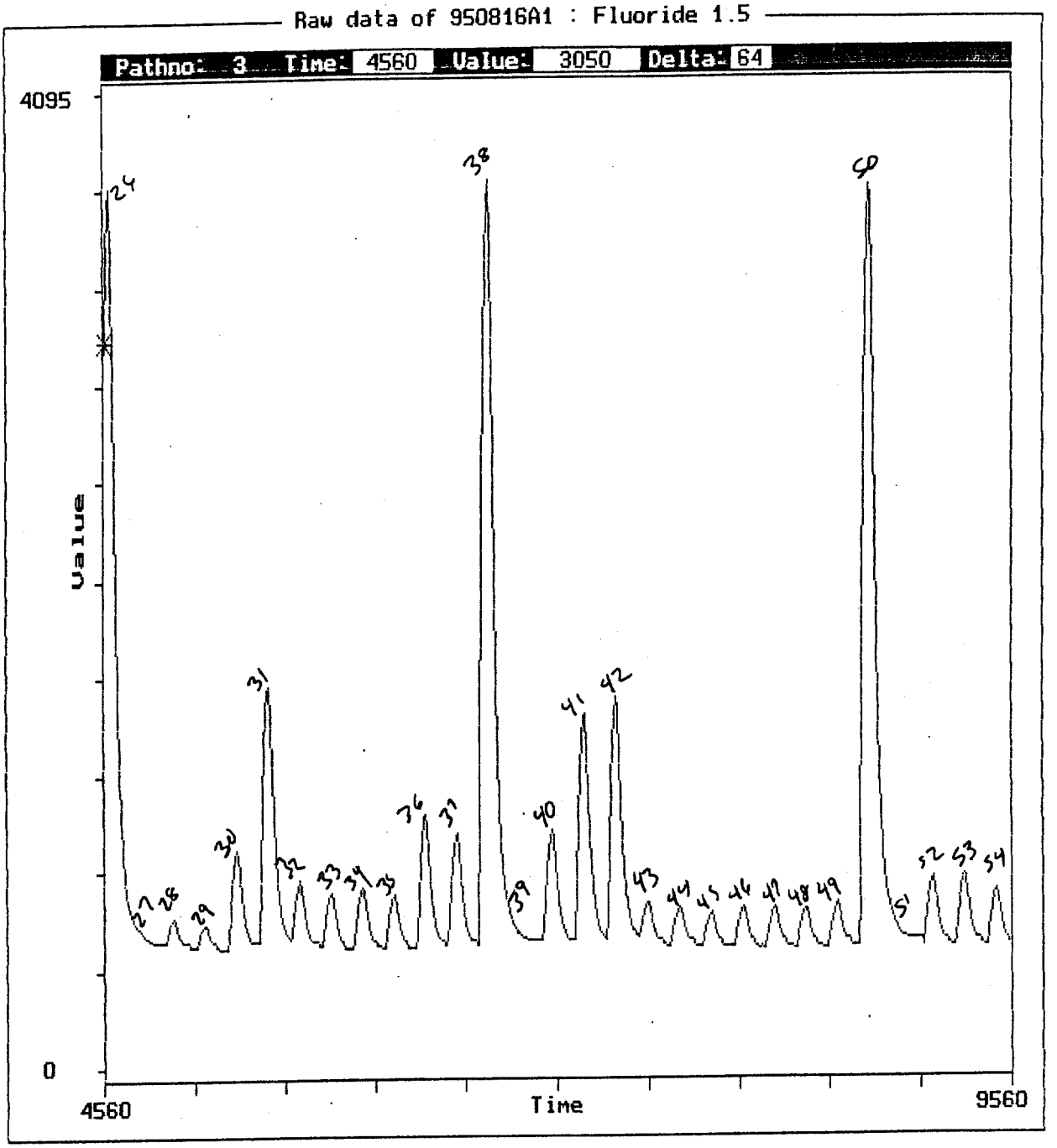
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	F52987-1	3	0.095	9486	4	0.0517	0
55	u	SPK 62-1	3	0.127	9662	4	0.1051	0
56	u	SPK 250-1	3	0.257	9838	4	0.3017	0
57	d	Drift	3	1.503	10012	4	1.8831	0
58	w	Wash	3	0.070	10247	4	0.0091	0
wt	rw	RunOut Wash	3	0.070	10487	4	0.0091	0


Calibration curve of 950816A1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0101901
Delta: 64
0.2692961
0.0091180
Concentration
0
Measured
900
Order : 2
r : 0.99717

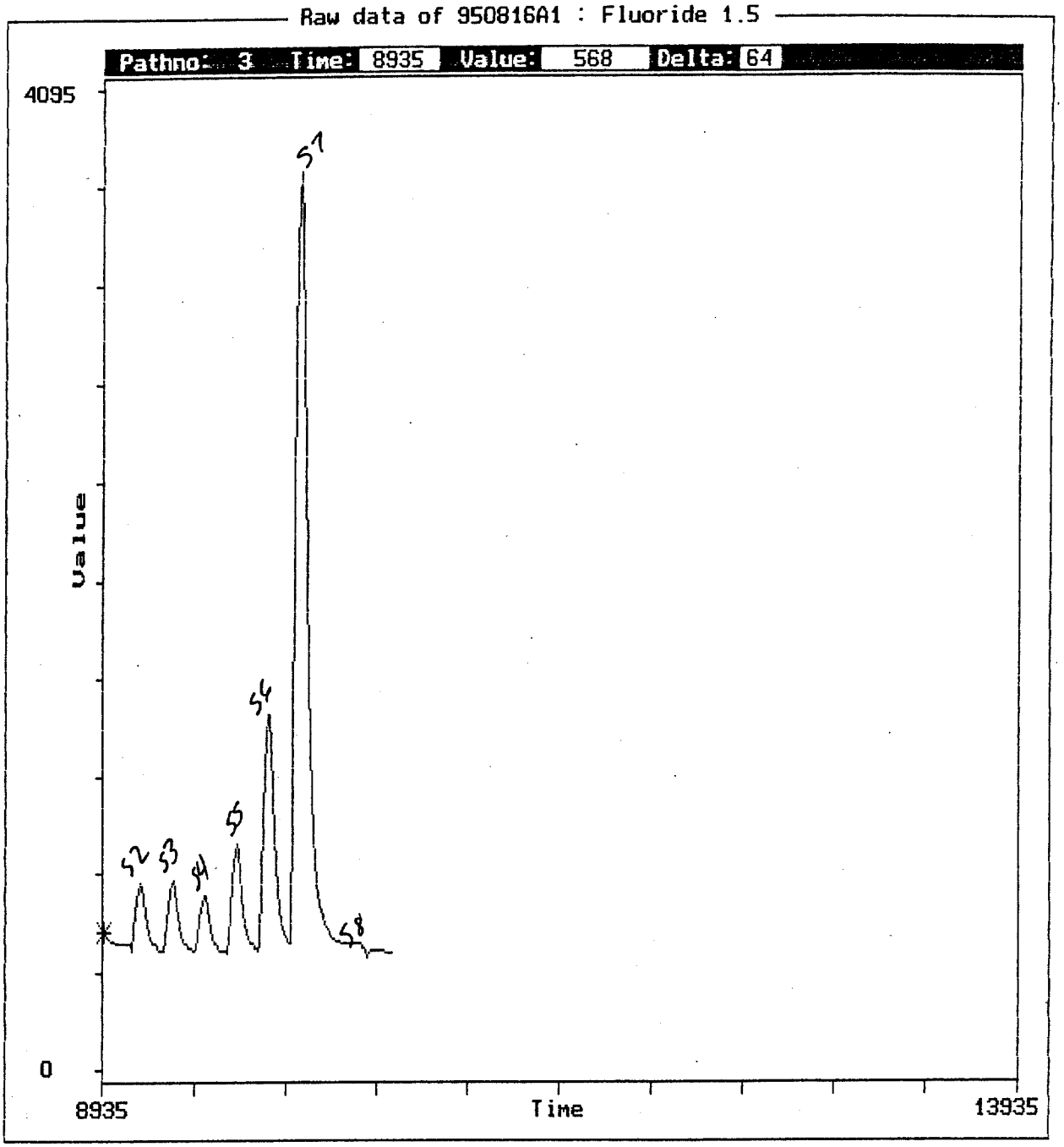




Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

DDW 8/25/9
AMDT 207
HWD 6329
Serum Curve 2

1995-03-21 12:30 OutPut of : 950821A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-21 07:55

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00071
a0 = -1.20061

Fluoride L
Calibration order = 2

Correlation : r = 0.99953

Result = a2 * x^2 + a1 * x + a0

a2 = -0.00000
a1 = 0.00025
a0 = 0.00771

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

1995-08-21 12:30 OutPut of : 950821A1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

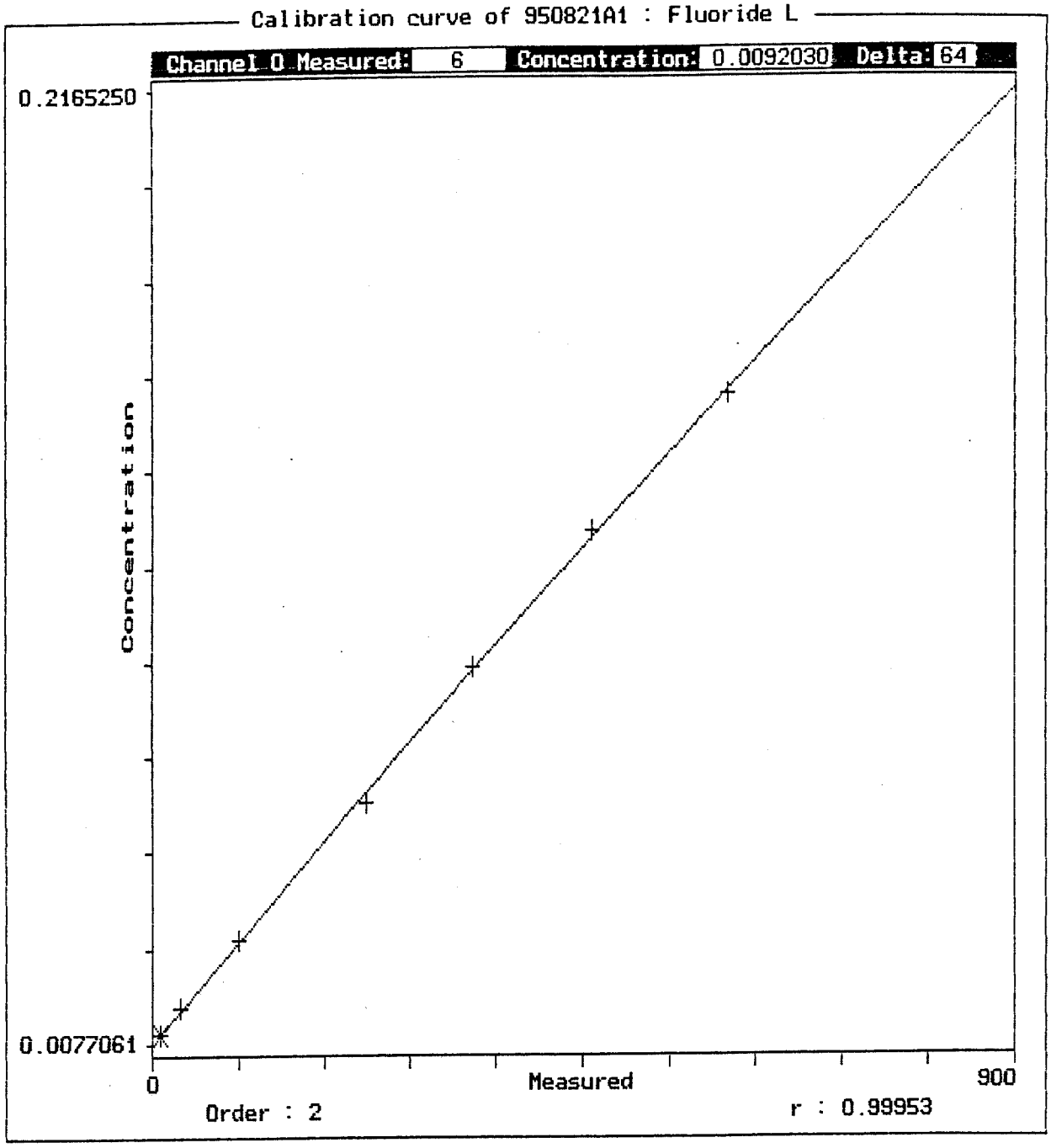
Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

1995-08-21 12:30 OutPut of : 950821A1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.063	65	4	0.0077	0
1	t	Tracer	3	1.468	208	4	0.5962	0
2	d	Drift	3	1.482	382	4	0.5992	0
3	w	Wash	3	0.063	570	4	0.0077	0
4	s1	Standard 1	3	0.066	728	4	0.0144	0
5	s2	Standard 2	3	0.073	903	4	0.0298	0
6	s3	Standard 3	3	0.090	1083	4	0.0624	0
7	s4	Standard 4	3	0.106	1259	4	0.0891	0
8	s5	Standard 5	3	0.128	1435	4	0.1184	0
9	s6	Standard 6	3	0.156	1609	4	0.1509	0
10	s7	Standard 7	3	0.279	1783	4	0.2480	0
11	s8	Standard 8	3	0.619	1958	4	0.3935	0
12	s9	Standard 9	3	1.224	2133	4	0.5438	0
13	s10	Standard 10	3	1.471	2309	4	0.5968	0
14	d	Drift	3	1.539	2483	4	0.6121	0
15	w	Wash	3	0.063	2723	4	0.0077	0
16	u	SERUM BLK 1	3	0.098	2832	4	0.0765	0
17	u	SERUM BLK 2	3	0.092	3012	4	0.0672	0
18	u	SERUM BLK 3	3	0.086	3184	4	0.0559	0
19	u	F52988-1	3	0.083	3360	4	0.0510	0
20	u	F52995-1	3	0.081	3534	4	0.0462	0
21	u	F52972-1	3	0.084	3710	4	0.0518	0
22	u	F52973-1	3	0.082	3885	4	0.0488	0
23	u	F52979-1	3	0.083	4060	4	0.0508	0
24	u	F52975-1	3	0.080	4234	4	0.0440	0
25	u	F52976-1	3	0.079	4410	4	0.0420	0
26	d	Drift	3	1.529	4583	4	0.6099	0
27	w	Wash	3	0.063	4810	4	0.0077	0
28	u	F52983-1	3	0.082	4933	4	0.0481	0
29	u	SPK 40-1	3	0.092	5111	4	0.0672	0
30	u	SPK 100-1	3	0.147	5285	4	0.1414	0
31	u	BLK-1	3	0.075	5460	4	0.0347	0
32	u	BLK-2	3	0.074	5632	4	0.0322	0
33	u	BLK-3	3	0.070	5810	4	0.0246	0
34	u	SPK 40-1	3	0.091	5988	4	0.0643	0
35	u	SPK 40-2	3	0.089	6162	4	0.0617	0
36	u	SPK 100-1	3	0.133	6336	4	0.1251	0
37	u	SPK 100-2	3	0.131	6512	4	0.1221	0
38	d	Drift	3	1.540	6684	4	0.6123	0
39	w	Wash	3	0.063	6902	4	0.0077	0
40	u	SPK 100-3	3	0.142	7037	4	0.1352	0
41	u	BLK	3	0.075	7207	4	0.0347	0
42	u	F52972-8	3	0.071	7384	4	0.0256	0
43	u	F52973-8	3	0.064	7558	4	0.0090	0
44	u	F52979-8	3	0.077	7736	4	0.0388	0
45	u	F52975-8	3	0.075	7909	4	0.0347	0
46	u	F52976-8	3	0.071	8085	4	0.0261	0
47	u	F52983-8	3	0.070	8257	4	0.0231	0
48	u	F52986-8	3	0.071	8437	4	0.0271	0
49	u	F52990-8	3	0.070	8609	4	0.0231	0
50	d	Drift	3	1.525	8785	4	0.6090	0
51	w	Wash	3	0.063	9012	4	0.0077	0
52	u	F52997-8	3	0.073	9136	4	0.0303	0
53	u	F52982-8	3	0.070	9310	4	0.0248	0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SPK 62-1	3	0.085	9488	4	0.0549	0
55	u	SPK 62-2	3	0.124	9664	4	0.1135	0
56	u	SPK 124-1	3	0.144	9838	4	0.1373	0
57	u	SPK 124-2	3	0.171	10014	4	0.1659	0
58	u	BLK	3	0.116	10189	4	0.1037	0
59	u	F52994-8	3	0.109	10365	4	0.0927	0
60	u	F53410-8	3	0.085	10539	4	0.0532	0
61	u	F52984-8	3	0.084	10715	4	0.0527	0
62	d	Drift	3	1.552	10888	4	0.6153	0
63	w	Wash	3	0.063	11124	4	0.0077	0
64	u	F52992-8	3	0.082	11239	4	0.0479	0
65	u	F52996-8	3	0.091	11415	4	0.0646	0
66	u	F52997-8	3	0.102	11589	4	0.0823	0
67	u	F52989-8	3	0.090	11766	4	0.0629	0
68	u	F52993-8	3	0.082	11938	4	0.0488	0
69	u	F52978-8	3	0.078	12108	4	0.0410	0
70	u	F52980-8	3	0.080	12290	4	0.0452	0
71	u	SPK 40-1	3	0.098	12466	4	0.0756	0
72	u	SPK 124-1	3	0.166	12642	4	0.1606	0
73	u	BLK	3	0.106	12816	4	0.0894	0
74	d	Drift	3	1.536	12990	4	0.6115	0
75	w	Wash	3	0.063	13232	4	0.0077	0
76	u	F52991-8	3	0.089	13341	4	0.0612	0
77	u	F52987-8	3	0.085	13517	4	0.0544	0
78	u	F52988-8	3	0.079	13683	4	0.0420	0
79	u	F52995-8	3	0.079	13867	4	0.0432	0
80	u	F52972-15	3	0.080	14042	4	0.0442	0
81	u	F52973-15	3	0.093	14218	4	0.0679	0
82	u	F52979-15	3	0.083	14392	4	0.0501	0
83	u	F52975-15	3	0.085	14566	4	0.0547	0
84	u	F52976-15	3	0.078	14740	4	0.0410	0
85	u	F52983-15	3	0.080	14916	4	0.0454	0
86	d	Drift	3	1.574	15092	4	0.6204	0
87	w	Wash	3	0.063	15334	4	0.0077	0
88	u	SPK 40-1	3	0.098	15441	4	0.0761	0
89	u	SPK 124-1	3	0.165	15619	4	0.1601	0
90	d	Drift	3	1.534	15792	4	0.6110	0
91	w	Wash	3	0.063	16032	4	0.0077	0
wt	rw	RunOut Wash	3	0.063	16267	4	0.0077	0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Channel 0 Measured: 6
Concentration: 0.0092030
Delta: 64
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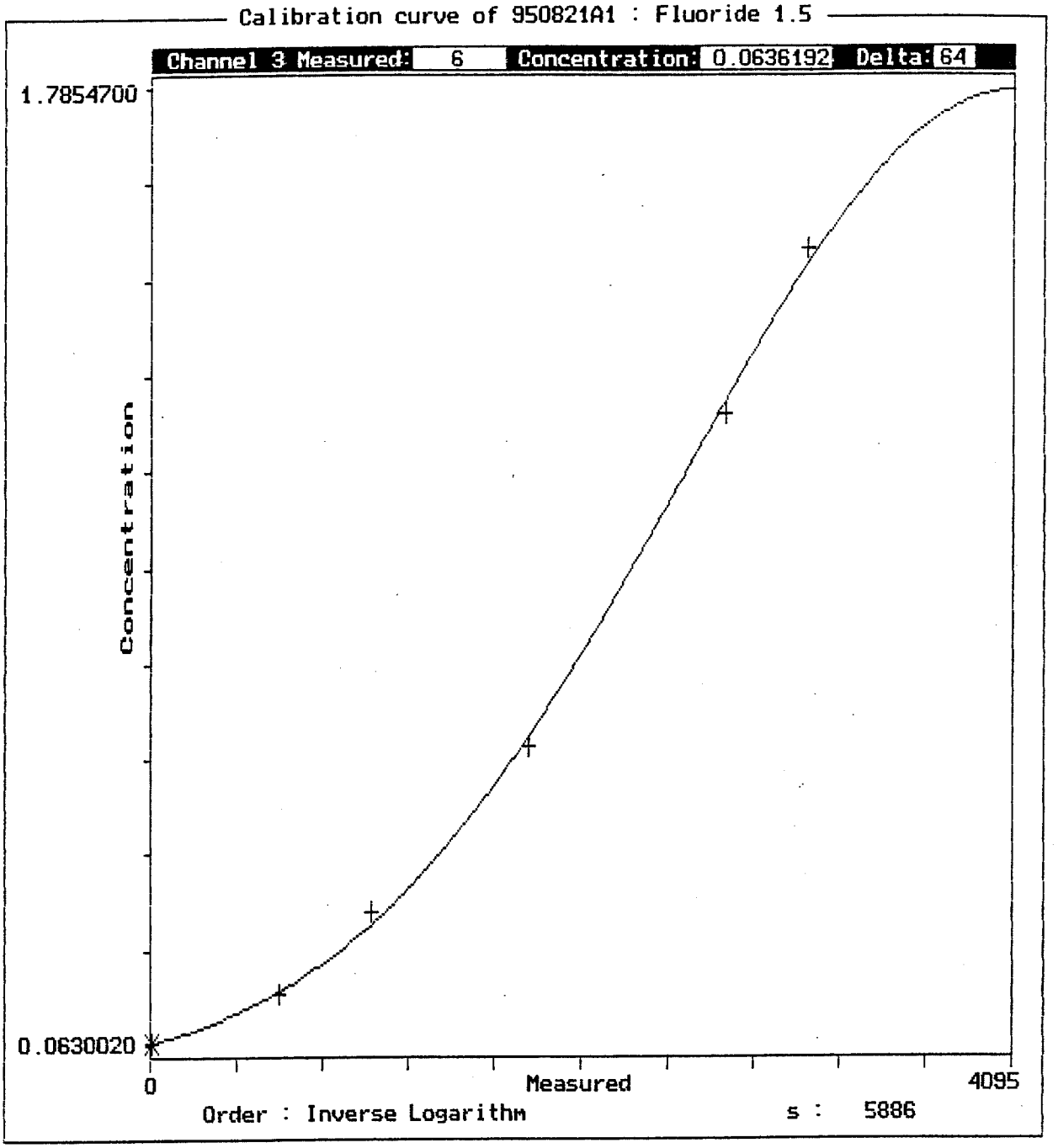
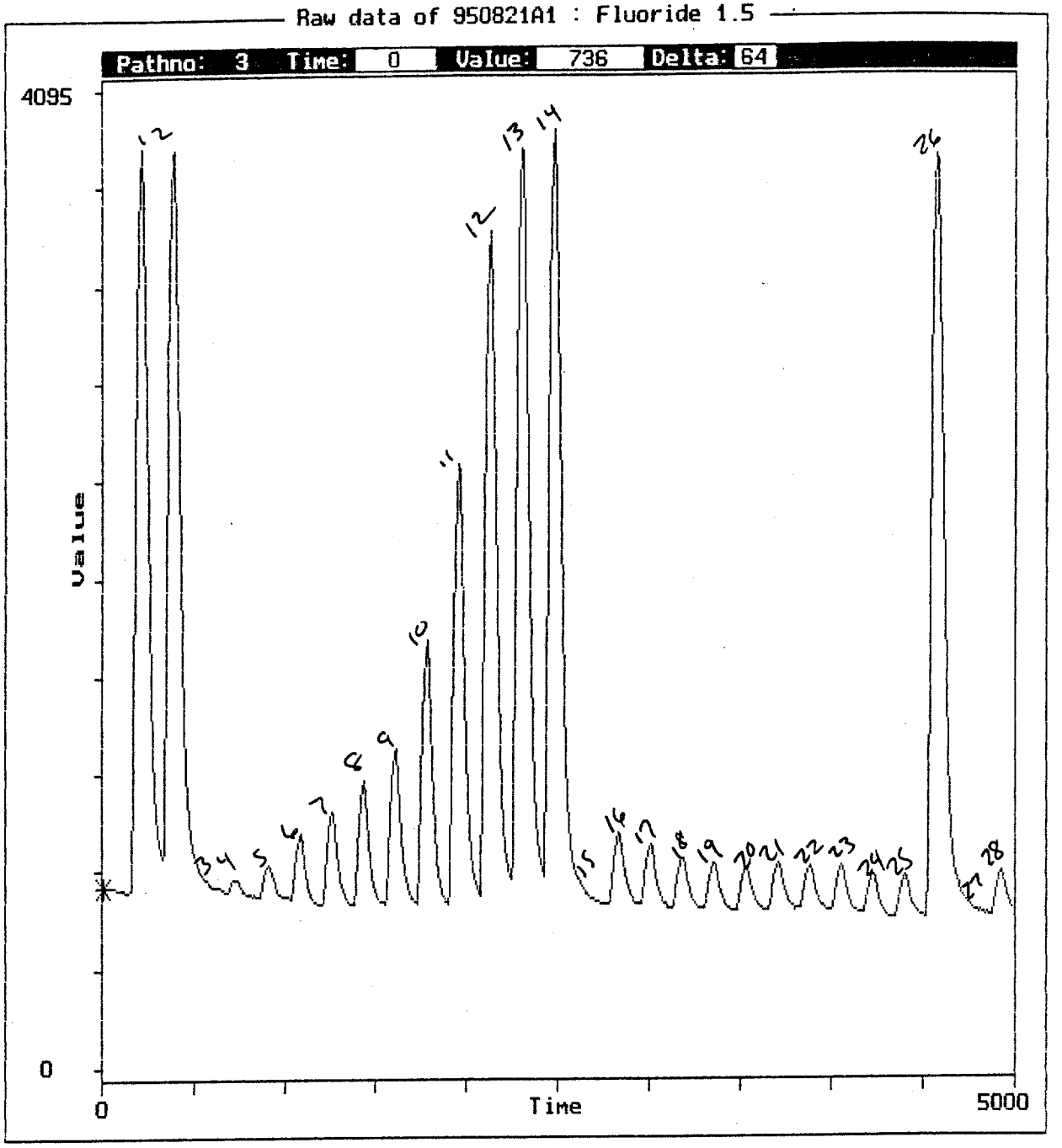
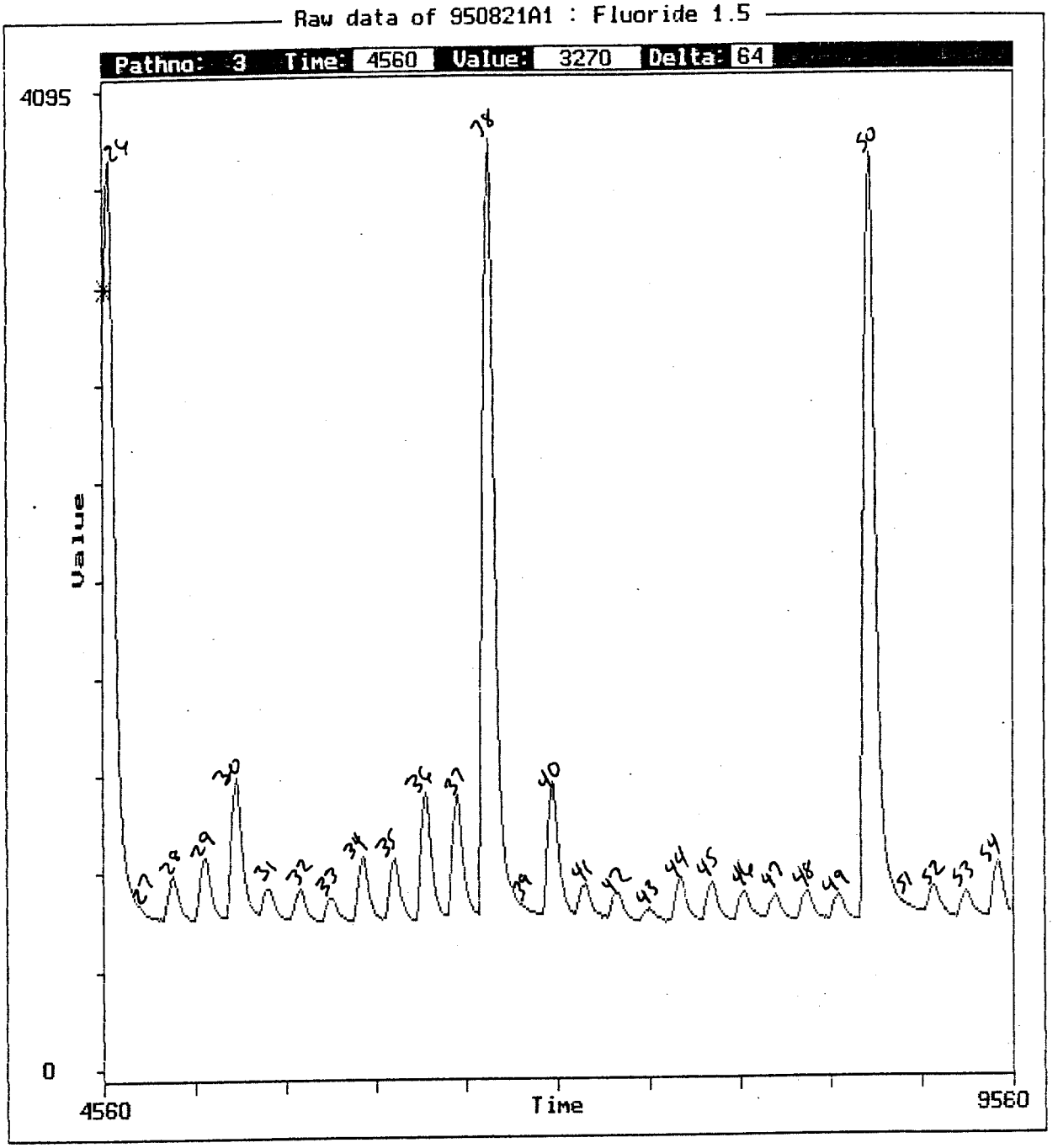
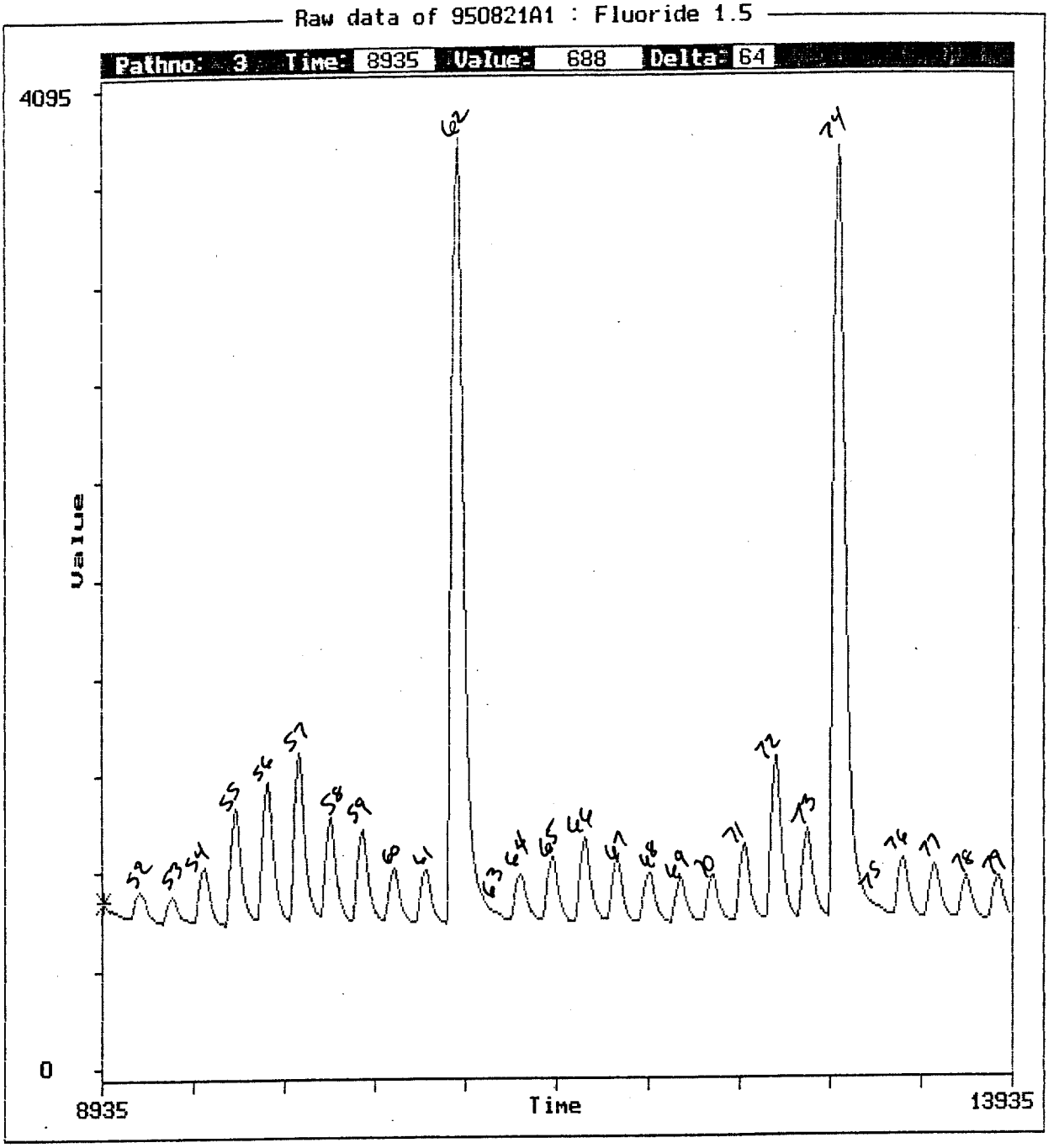
Concentration
0
Measured
900
Order : 2
r : 0.99953


Calibration curve of 950821A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0636192
Delta: 64
1.7854700
0.0630020
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5886

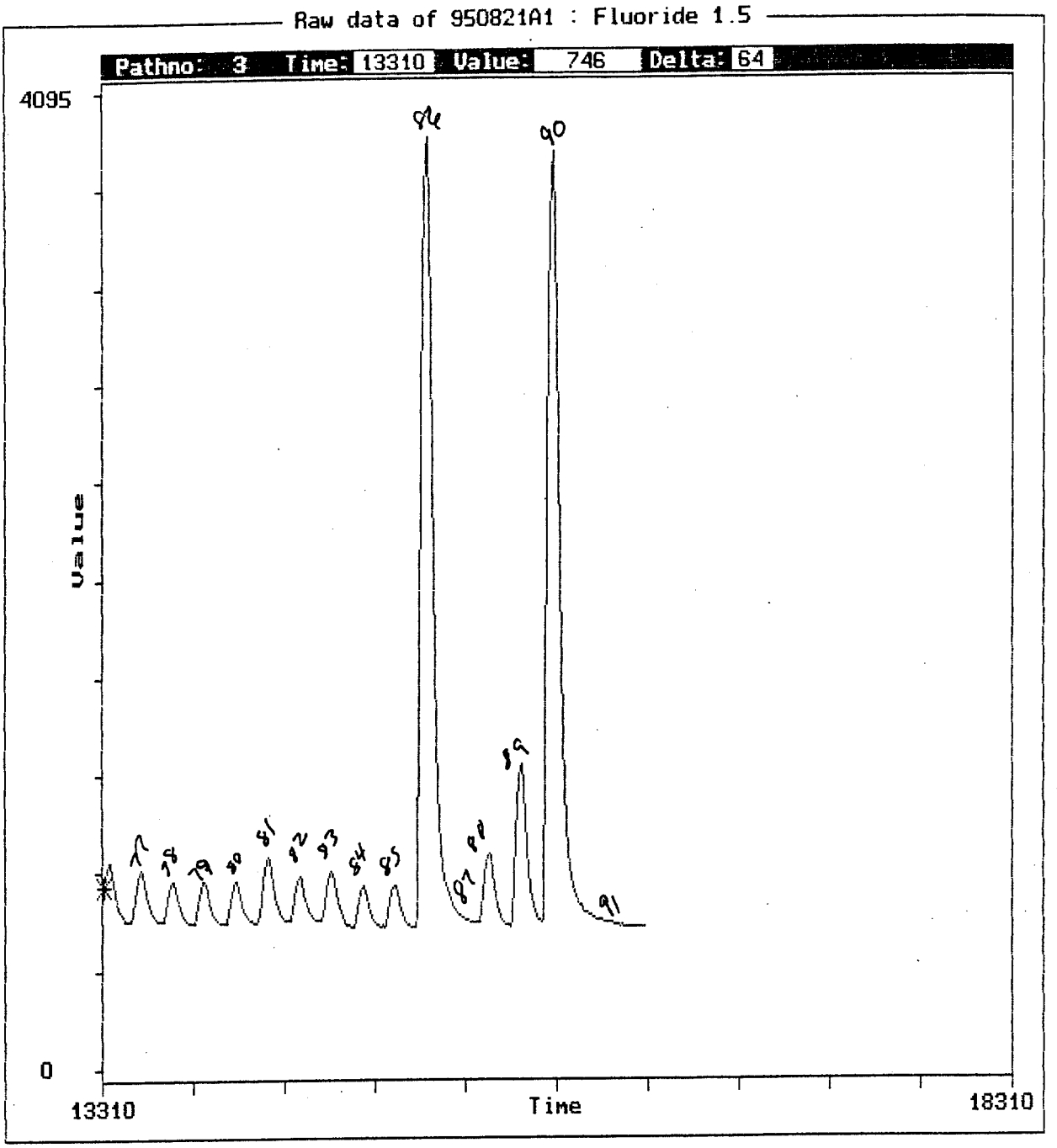


Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦


Raw data of 950821A1 : Fluoride 1.5
Pathno: 3 Time: 4560 Value: 3270 Delta: 64
4095
0
Value
4560
Time
9560
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

DDW 8/25/95
AMDT 20795.1
HWI 6329-135
Serum Curve 2-1

1995-08-21 16:58 OutPut of : 950821B1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-21 12:29

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821B1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00068
a0 = -1.19997

Fluoride L
Calibration order = 2

Correlation : r = 0.99960

Result = a2 * x^2 + a1 * x + a0

a2 = -0.00000
a1 = 0.00025
a0 = 0.00307

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

1995-08-21 16:58 OutPut of : 950821B1

Fluoride 1.5

Path number	: 3
Signal type	: Debubbled
Decolor	: Yes
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off
s1 sTandard	: Ignore
s2 sTandard	: Ignore
s3 sTandard	: Ignore
s4 sTandard	: Ignore
s5 sTandard	: Ignore
s6 sTandard	: 0.150
s7 sTandard	: 0.300
s8 sTandard	: 0.600
s9 sTandard	: 1.200
s10 sTandard	: 1.500
Order	: Inverse Logarithm
Dimension	: PPM
start Value	: 500 DU
trigger Limit	: 1800 Sec
Peak shape	: Pointed
stArt ignore	: 60 Sec
eNd ignore	: 120 Sec
Measure window	: 75 %
Filter	: No
Regeneration	: No
formUla	:
output	: ##.###

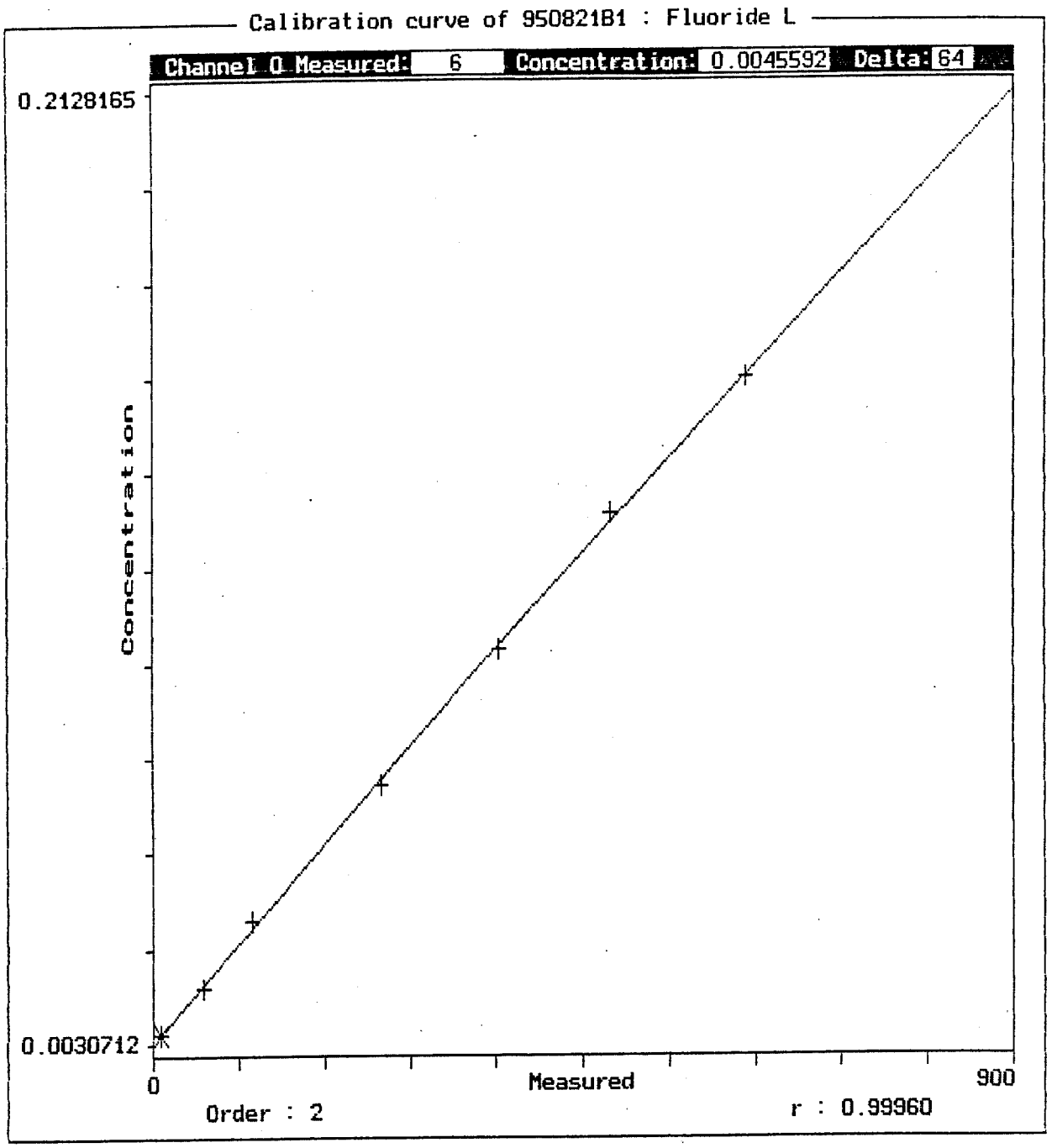
Fluoride L

Path number	: 0
Signal type	: Debubbled
Decolor	: No
system Number	: 0
diLute	: No
Resample	: No
dil Threshold	: 4095
diG output	: 0
Window event	: Off

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.063	65	4	0.0031	0
1	t	Tracer	3	1.488	208	4	0.6348	0
2	d	Drift	3	1.478	384	4	0.6322	0
3	w	Wash	3	0.063	625	4	0.0031	0
4	s1	Standard 1	3	0.068	733	4	0.0157	0
5	s2	Standard 2	3	0.074	911	4	0.0284	0
6	s3	Standard 3	3	0.091	1085	4	0.0612	0
7	s4	Standard 4	3	0.109	1261	4	0.0909	0
8	s5	Standard 5	3	0.129	1435	4	0.1181	0
9	s6	Standard 6	3	0.156	1611	4	0.1507	0
10	s7	Standard 7	3	0.279	1787	4	0.2517	0
11	s8	Standard 8	3	0.620	1960	4	0.4063	0
12	s9	Standard 9	3	1.224	2135	4	0.5703	0
13	s10	Standard 10	3	1.472	2311	4	0.6308	0
14	d	Drift	3	1.435	2485	4	0.6213	0
15	w	Wash	3	0.063	2662	4	0.0031	0
16	u	SERUM BLK 1	3	0.075	2837	4	0.0314	0
17	u	SERUM BLK 2	3	0.070	3013	4	0.0184	0
18	u	SPK 40-1	3	0.080	3186	4	0.0406	0
19	u	SPK 40-2	3	0.081	3362	4	0.0431	0
20	u	SPK 40-3	3	0.086	3536	4	0.0515	0
21	u	SPK 124-1	3	0.145	3714	4	0.1381	0
22	u	SPK 124-2	3	0.150	3888	4	0.1441	0
23	u	SPK 124-3	3	0.145	4062	4	0.1386	0
24	u	BLK	3	0.076	4238	4	0.0326	0
25	u	F52986-15	3	0.067	4411	4	0.0127	0
26	d	Drift	3	1.408	4586	4	0.6146	0
27	w	Wash	3	0.063	4732	4	0.0031	0
28	u	F52990-15	3	0.069	4938	4	0.0164	0
29	u	F52997-15	3	0.067	5109	4	0.0115	0
30	u	F52982-15	3	0.064	5285	4	0.0058	0
31	u	F52994-15	3	0.069	5459	4	0.0167	0
32	u	F53410-15	3	0.068	5635	4	0.0137	0
33	u	F52984-15	3	0.065	5811	4	0.0070	0
34	u	F52992-15	3	0.065	5987	4	0.0083	0
35	u	F52996-15	3	0.066	6163	4	0.0090	0
36	u	F52977-15	3	0.066	6334	4	0.0110	0
37	u	SPK 40-1	3	0.081	6513	4	0.0431	0
38	d	Drift	3	1.434	6686	4	0.6211	0
39	w	Wash	3	0.063	6924	4	0.0031	0
40	u	SPK 124-1	3	0.191	7036	4	0.1851	0
41	u	BLK	3	0.097	7212	4	0.0715	0
42	u	F52989-15	3	0.118	7386	4	0.1036	0
43	u	F52993-15	3	0.088	7559	4	0.0561	0
44	u	F52978-15	3	0.089	7736	4	0.0585	0
45	u	F52980-15	3	0.088	7911	4	0.0559	0
46	u	F52991-15	3	0.086	8083	4	0.0530	0
47	u	F52987-15	3	0.083	8263	4	0.0469	0
48	u	F52988-15	3	0.083	8437	4	0.0474	0
49	u	F52995-15	3	0.079	8613	4	0.0385	0
50	d	Drift	3	1.454	8787	4	0.6263	0
51	w	Wash	3	0.063	9024	4	0.0031	0
52	u	F52972-22	3	0.083	9137	4	0.0457	0
53	u	F52973-22	3	0.085	9313	4	0.0511	0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SPK 40-1	3	0.117	9489	4	0.1017	0
55	u	SPK 40-2	3	0.093	9662	4	0.0655	0
56	u	SPK 124-1	3	0.146	9838	4	0.1391	0
57	u	BLK	3	0.090	10010	4	0.0593	0
58	u	F52979-22	3	0.080	10186	4	0.0411	0
59	u	F52975-22	3	0.077	10365	4	0.0341	0
60	u	F52976-22	3	0.076	10537	4	0.0324	0
61	u	F52983-22	3	0.082	10713	4	0.0453	0
62	d	Drift	3	1.419	10886	4	0.6175	0
63	w	Wash	3	0.063	11120	4	0.0031	0
64	u	F52986-22	3	0.082	11237	4	0.0438	0
65	u	F52990-22	3	0.077	11409	4	0.0343	0
66	u	F52997-22	3	0.078	11583	4	0.0363	0
67	u	F52982-22	3	0.074	11762	4	0.0284	0
68	u	F52994-22	3	0.082	11936	4	0.0445	0
69	u	F53410-22	3	0.083	12114	4	0.0460	0
70	u	SPK 40-1	3	0.108	12288	4	0.0893	0
71	u	SPK 124-1	3	0.168	12462	4	0.1627	0
72	u	SPK 62-1	3	0.131	12638	4	0.1211	0
73	u	BLK	3	0.091	12812	4	0.0621	0
74	d	Drift	3	1.469	12985	4	0.6301	0
75	w	Wash	3	0.063	13227	4	0.0031	0
76	u	F52984-22	3	0.083	13337	4	0.0469	0
77	u	F52992-22	3	0.082	13511	4	0.0455	0
78	u	F52996-22	3	0.084	13685	4	0.0479	0
79	u	F52977-22	3	0.083	13858	4	0.0467	0
80	u	F52989-22	3	0.082	14035	4	0.0440	0
81	u	F52993-22	3	0.079	14211	4	0.0397	0
82	u	SPK 40-1	3	0.106	14379	4	0.0857	0
83	u	SPK 124-1	3	0.152	14559	4	0.1459	0
84	d	Drift	3	1.461	14735	4	0.6280	0
85	w	Wash	3	0.063	14976	4	0.0031	0
wt	rw	RunOut Wash	3	0.063	15210	4	0.0031	0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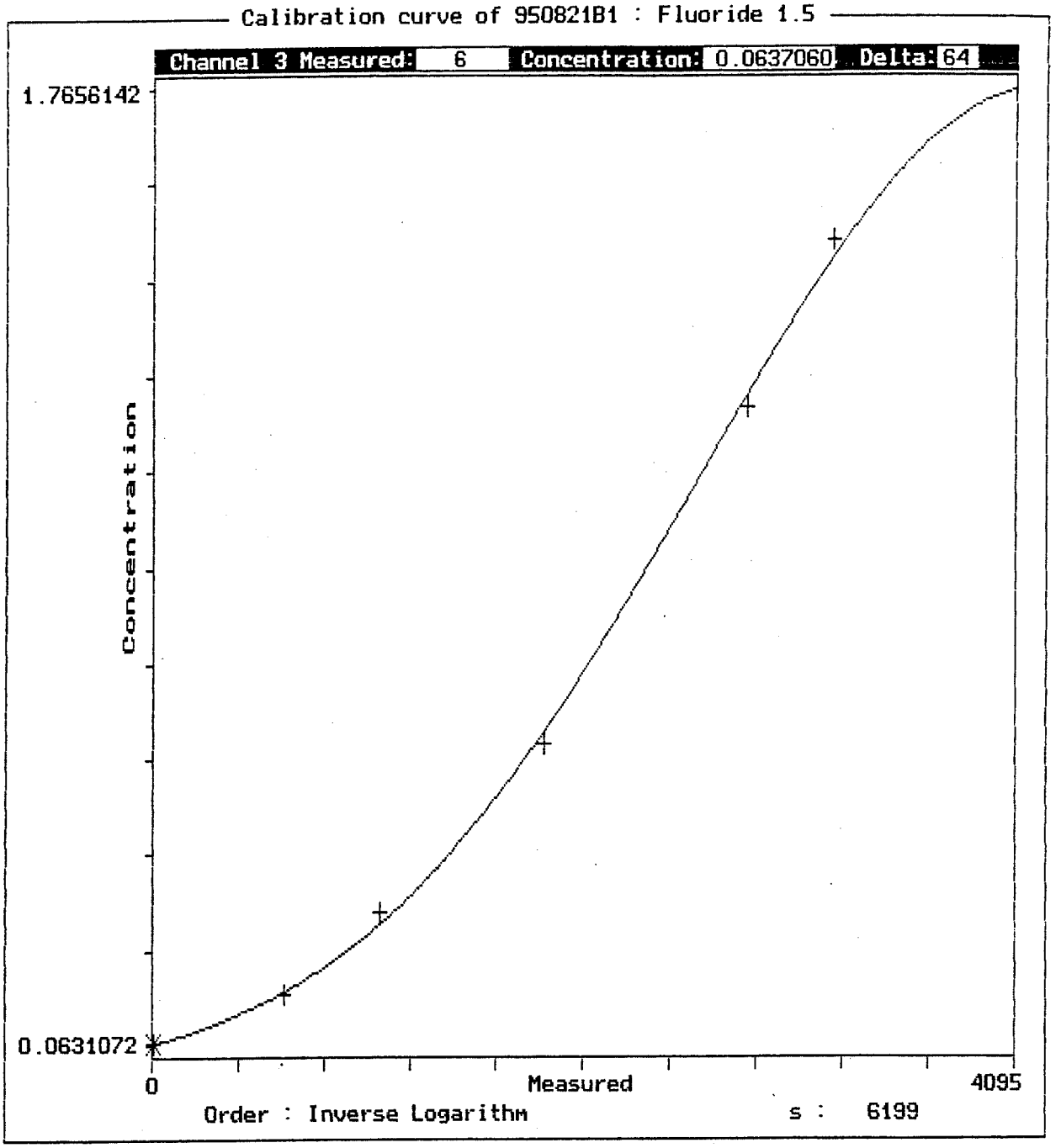
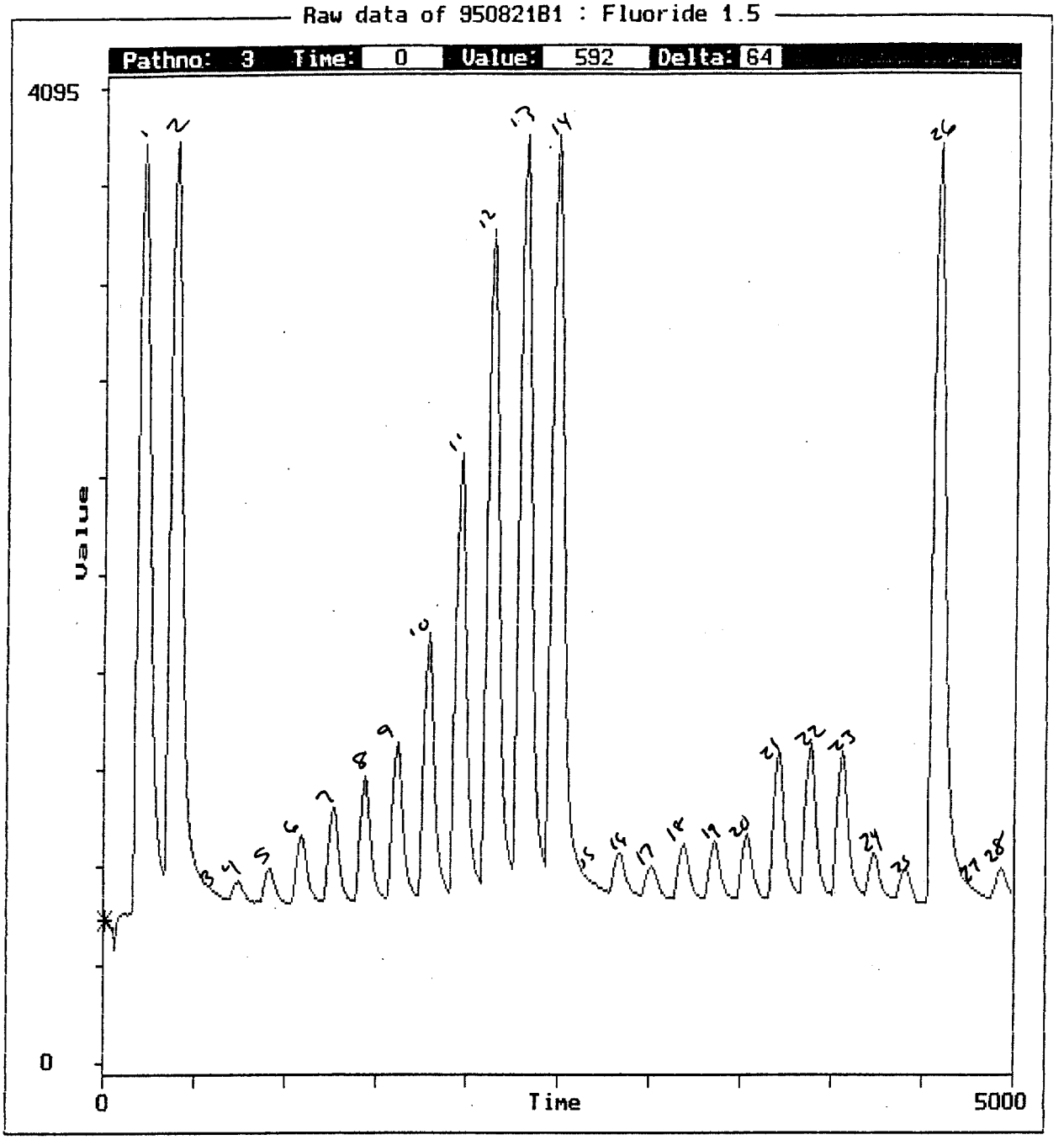
Channel 0 Measured: 6
Concentration: 0.0045592
Delta: 64
0.2128165
0.0030712
Concentration
0
Measured
900
Order : 2
r : 0.99960


Calibration curve of 950821B1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0637060
Delta: 64
1.7656142
0.0631072
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 6199


Raw data of 95082181 : Fluoride 1.5
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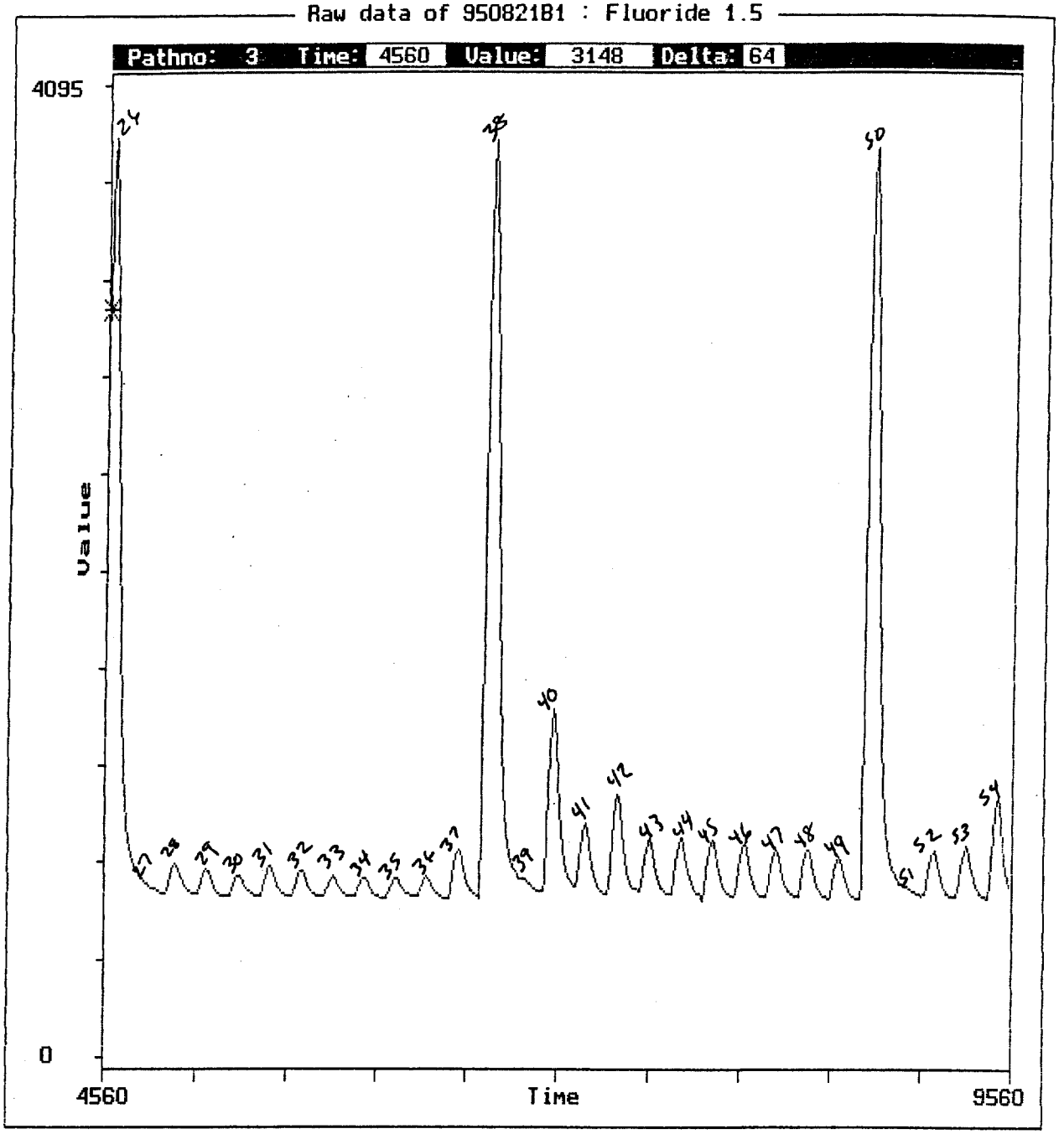
4095
0
Value
0
Time
5000
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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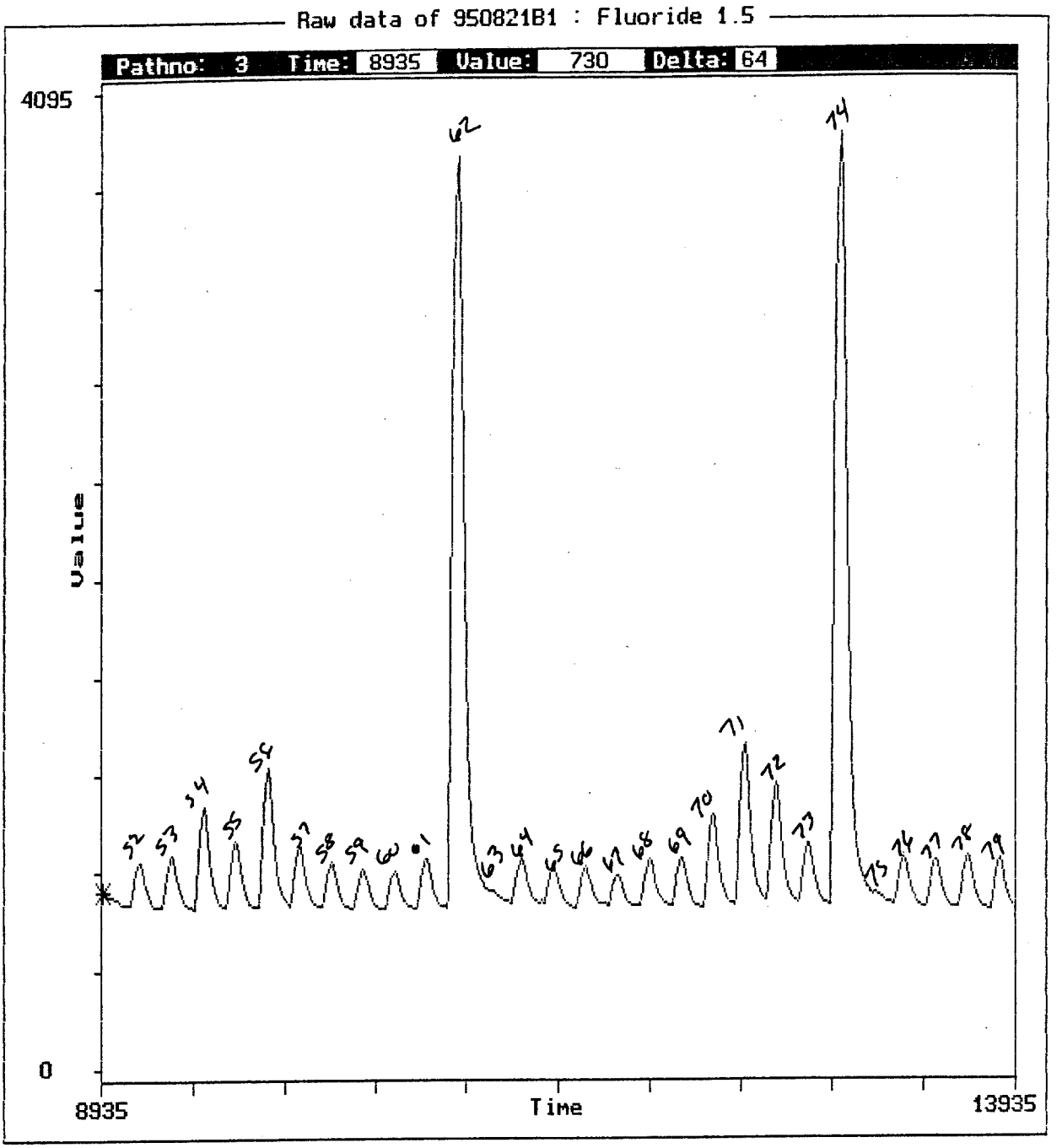

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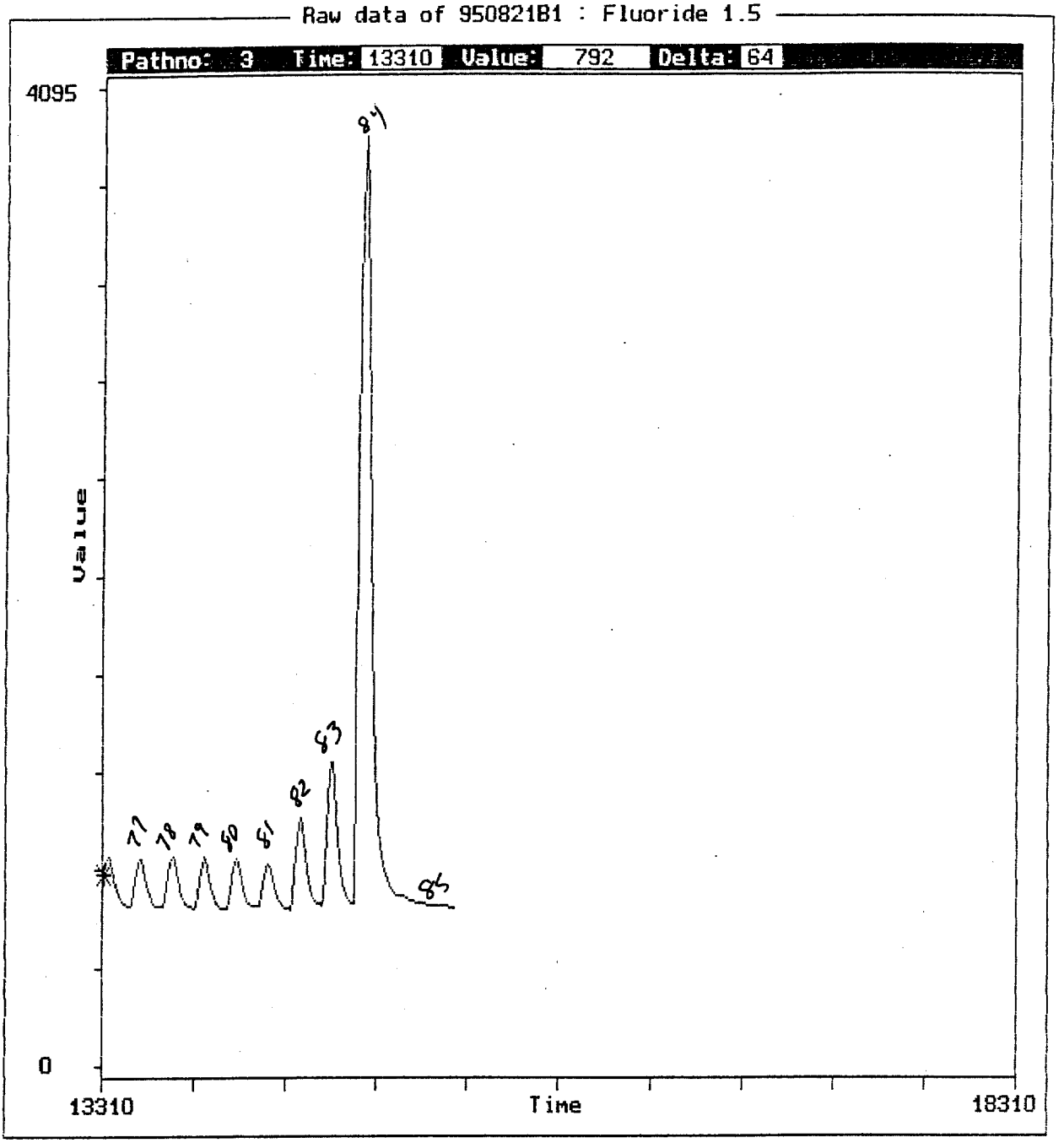


Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

Printout of Sample Table : 6329135D Inserted in System Table of : group 1

0	iw	Initial Wash	1	1.0000
1	t	Tracer	1	1.000
2	d	Drift	1	1.000
3	w	Wash	1	1.000
4	s1	Standard 1	1	1.000
5	s2	Standard 2	1	1.000
6	s3	Standard 3	1	1.000
7	s4	Standard 4	1	1.000
8	s5	Standard 5	1	1.000
9	s6	Standard 6	1	1.000
10	s7	Standard 7	1	1.000
11	s8	Standard 8	1	1.000

000793

DDW 8/25/95 (D
MDT 20795.1
HWI 6329-135
Serum Cmmn 2 d

1995-08-22 09:05 OutPut of : 950822A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-22 06:49

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950822A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00075
a0 = -1.18734

Fluoride L
Calibration order = 2

Correlation : r = 0.99917

Result = a2 * x² + a1 * x + a0

a2 = -0.00000
a1 = 0.00027
a0 = 0.00577

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

1995-08-22 09:05 OutPut of : 950822A1

Fluoride 1.5

```
Path number    :  3
Signal type    : Debubbled
Decolor        : Yes
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off

s1   sTandard  : Ignore
s2   sTandard  : Ignore
s3   sTandard  : Ignore
s4   sTandard  : Ignore
s5   sTandard  : Ignore
s6   sTandard  :    0.150
s7   sTandard  :    0.300
s8   sTandard  :    0.600
s9   sTandard  :    1.200
s10  sTandard  :    1.500
Order : Inverse Logarithm
Dimension : PPM
start Value    : 500  DU
trigger Limit  : 1800 Sec
Peak shape     : Pointed
stArt ignore   : 60   Sec
eNd   ignore   : 120  Sec
Measure window : 75   %
Filter         : No
Regeneration   : No
formUla   :
output    : ##.###
```

Fluoride L

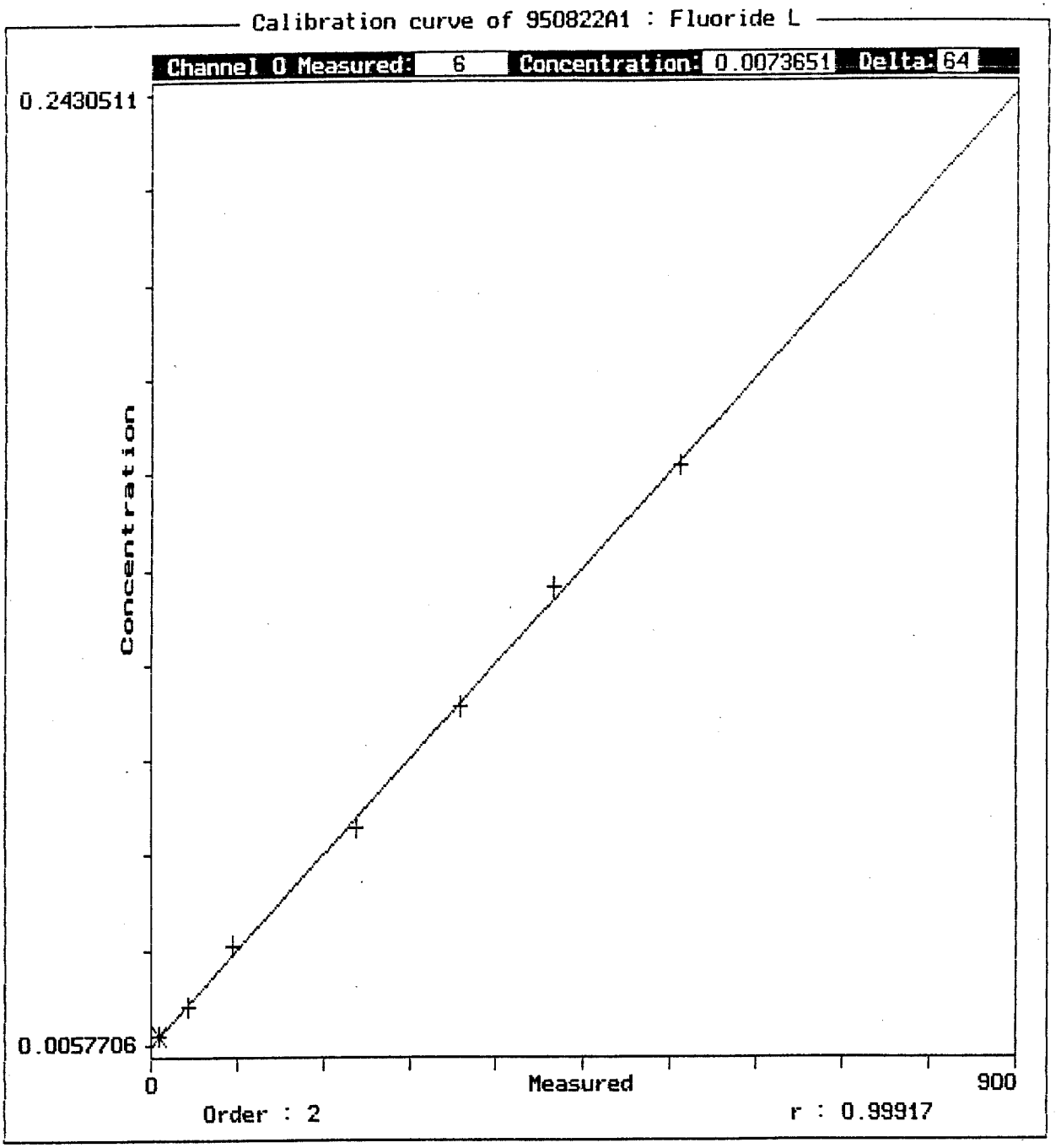
```
Path number    :  0
Signal type    : Debubbled
Decolor        : No
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off
```

1995-08-22 09:05 OutPut of : 950822A1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L			
			PPM			*PPM*			
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F	Time
wt	iw	Initial Wash	3	0.065	65	4	0.0058		0
1	t	Tracer	3	1.461	208	4	0.7660		0
2	d	Drift	3	1.475	383	4	0.7723		0
3	w	Wash	3	0.065	616	4	0.0058		0
4	s1	Standard 1	3	0.069	729	4	0.0156		0
5	s2	Standard 2	3	0.075	907	4	0.0281		0
6	s3	Standard 3	3	0.093	1083	4	0.0623		0
7	s4	Standard 4	3	0.111	1259	4	0.0911		0
8	s5	Standard 5	3	0.129	1433	4	0.1167		0
9	s6	Standard 6	3	0.157	1609	4	0.1512		0
10	s7	Standard 7	3	0.277	1785	4	0.2587		0
11	s8	Standard 8	3	0.621	1959	4	0.4443		0
12	s9	Standard 9	3	1.227	2134	4	0.6732		0
13	s10	Standard 10	3	1.469	2309	4	0.7697		0
14	d	Drift	3	1.536	2484	4	0.8009		0
15	w	Wash	3	0.065	2722	4	0.0058		0
16	u	SERUM BLK 1	3	0.085	2837	4	0.0472		0
17	u	SERUM BLK 2	3	0.078	3011	4	0.0334		0
18	u	SPK 40-1	3	0.083	3187	4	0.0432		0
19	u	SPK 40-2	3	0.090	3361	4	0.0570		0
20	u	SPK 40-3	3	0.090	3533	4	0.0575		0
21	u	SPK 40-4	3	0.092	3710	4	0.0609		0
22	u	SPK 124-1	3	0.105	3886	4	0.0829		0
23	u	SPK 124-2	3	0.138	4059	4	0.1283		0
24	u	SPK 124-3	3	0.278	4236	4	0.2595		0
25	u	SPK 124-4	3	0.129	4412	4	0.1172		0
26	d	Drift	3	1.533	4584	4	0.7994		0
27	w	Wash	3	0.065	4825	4	0.0058		0
28	u	SPK 100-1	3	0.121	4936	4	0.1059		0
29	u	SPK 100-2	3	0.141	5112	4	0.1317		0
30	u	SPK 100-3	3	0.137	5286	4	0.1278		0
31	u	BLK	3	0.101	5460	4	0.0755		0
32	u	BLK	3	0.080	5635	4	0.0390		0
33	u	F52978-22	3	0.078	5811	4	0.0334		0
34	u	F52980-22	3	0.082	5987	4	0.0424		0
35	u	F52991-22	3	0.076	6151	4	0.0291		0
36	u	F52987-22	3	0.073	6335	4	0.0249		0
37	u	F52988-22	3	0.070	6511	4	0.0169		0
38	d	Drift	3	1.532	6685	4	0.7989		0
39	w	Wash	3	0.065	6927	4	0.0058		0
40	u	F52995-22	3	0.074	7037	4	0.0268		0
41	u	~~SPK 40-1~~	~~3~~	~~0.062~~	~~A 7210~~	~~4~~	~~#.####~~		~~0~~
42	u	SPK 100-1	3	0.121	7386	4	0.1059		0
43	d	Drift	3	1.550	7561	4	0.8082		0
44	w	Wash	3	0.065	7803	4	0.0058		0
wt	rw	RunOut Wash	3	0.065	8036	4	0.0058		0

Blank T1S1B - sample 8/22/95 000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Channel 0 Measured: 6
Concentration: 0.0073651
Delta: 64
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0.0057706
Concentration
0
Measured
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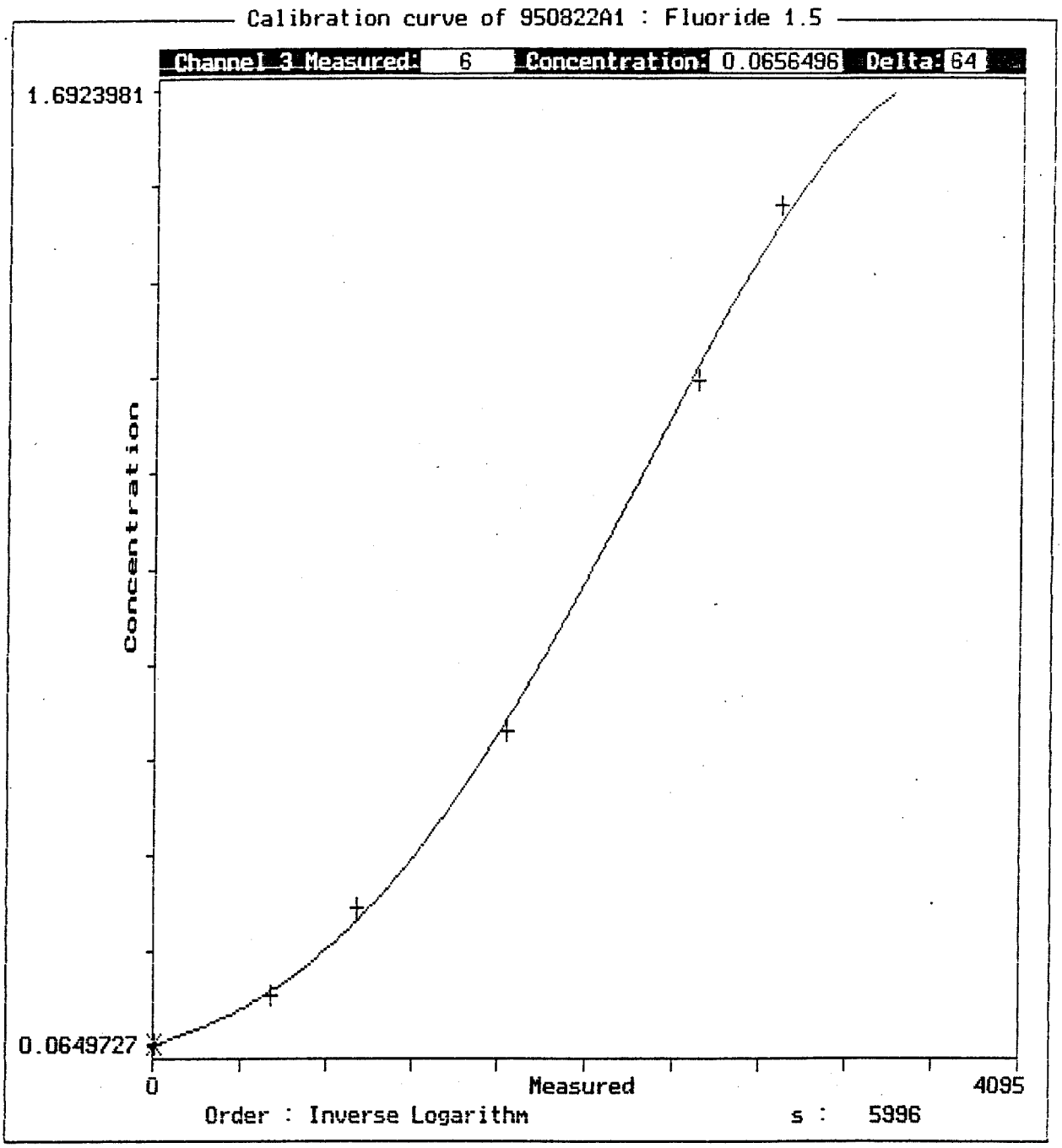
Order : 2
r : 0.99917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Channel 3 Measured: 6
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Concentration
0.0649727
0
Measured
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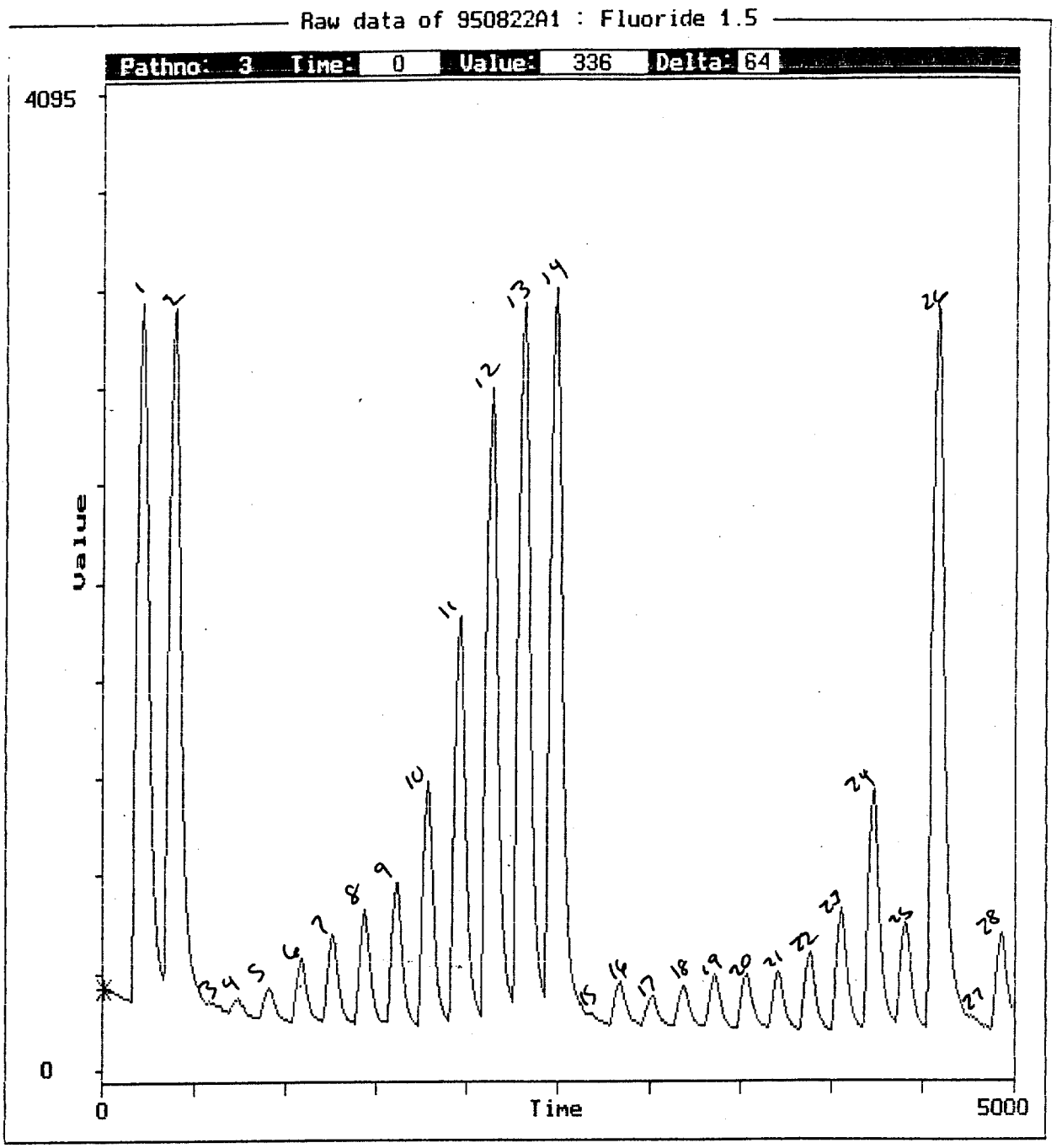
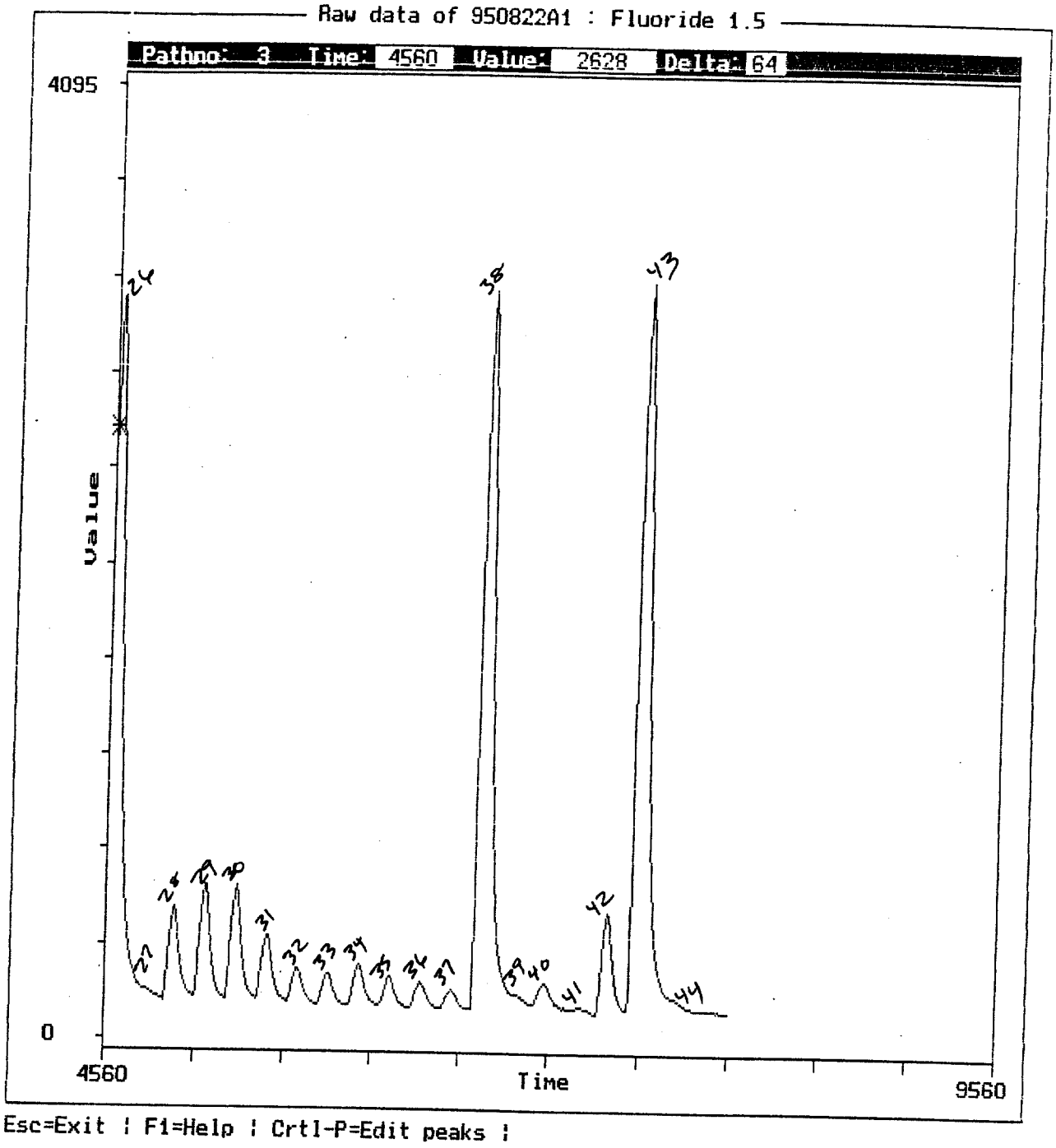
Order : Inverse Logarithm
s : 5996



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦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4095
0
Value
4560
Time
9560
24
38
43
42
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-134

Study Number: AMDT-111694.1
Test Substance: FC-120 (T-6052)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

The liver samples at 48 hours after single intravenous administration of FC-120 (T-6052) were analyzed by combustion for total organic fluorine. T-6052 is a 0.02% solution of FC-120. Only the 200 mg/kg (10 ug/kg) and 1000 mg/kg (50 ug/kg) samples had detectable organic fluorine: 48 and 106 ug/whole liver, respectively. These trivial amounts of organic fluorine are a reflection of the low doses. There is a marker for dermal absorption studies, if the doses are higher than used in this study.

2.0 INTRODUCTION

The liver and serum samples from HWI#6329-134 were available for analysis. This compound is perfluorodecanesulfonate (ammonium salt). There is not expected to be any biotransformation of this compound and the pharmacokinetics and disposition are expected to be similar to that found for perfluorooctanesulfonate. The tissues at 48 hours were analyzed by combustion for total organic fluorine and by electrospray mass spectrometry for perfluorodecanesulfonate anion. The data were to be analyzed to provide data for the assessment of a subsequent dermal absorption study. The high dose is just 50 ug/kg. T-6052 is a 0.02% solution of FC-120, which is 25% solids.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20 ± 10^{0} C.

3.6 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day absorption study, so all tissues will be retained.

4.0 EXPERIMENTAL - Overview

Serum and tissues from animals dosed as described (HWI#6329-134), were available for analysis for fluorine compounds. Since perfluorodecanesulfonate anion is not biotransformed, the analysis was accomplished with combustion and subsequent analysis for fluorine. The fluorine data are related directly to perfluorodecane-sulfonate anion concentration. Additional analysis of liver samples with electrospray mass spectrometry provides evidence that the perfluorodecanesulfonate anion is present. Data from these analysis will be used to assess the extent of dermal absorption in a subsequent study (HWI#6329-135).

5.0 EXPERIMENTAL - METHODS

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

5.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

6.0 DATA ANALYSIS

The data (Skalar) is attached for combustion analysis. The total organic fluorine in liver at 48 hours after an intravenous dose of FC-120 was nondetected for the control, 0.1 ug/kg, and 1.0 ug/kg groups. The total organic fluorine measured for

the 10 ug/kg and 50 ug/kg rabbits were 48 and 106 ug/whole liver, respectively. Electrospray mass spectrometry (see attached) confirmed the presence of perfluorodecanesulfonate (m/z=599).

Other data was collected using the Dorhman organic halide analyzer, Orion ion analyzer (liver and serum), Skalar segmented flow analyzer with ion selective electrode (serum), and electrospray mass spectrometry (liver) - see appendices. This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances that May Have Affected the Quality of the Data: The problem with this analysis is that there is not nearly enough fluorine in the liver after these intravenous doses because the doses are too low.

7.0 CONCLUSION

This pharmacokinetic study is not useful in terms of providing data for the assessment of a dermal absorption study. The perfluorodecanesulfonic acid anion expected is not observed in liver except for a trace at the highest dose (50 ug/kg). If the dermal absorption study doses are high enough, there is a marker.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-134, "Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-111694.1

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-111694.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.11.4 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.5 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.1.1 Final Report: HWI#6329-134, "Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 1, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-134

Phone 608-241-4471 Fax 608
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD. MADISON, WI 53704

QUALITY ASSURANCE STATEMENT

HWI 6329-134

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
11/05/94	11/05/94	Protocol Review	11/08/94	12/10/94
11/11/94	11/11/94	Animal Observation	11/11/94	12/10/94
01/10/95	01/10/95	Data/Report Review	01/10/95	02/10/95
01/30/95	01/30/95	Report Rereview	01/30/95	02/10/95

Cecilia M. Danner
Representative, Quality Assurance Unit

2/1/95
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

Test Material	T-6052
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Experimental Start Date	November 11, 1994
Experimental Termination Date	November 13, 1994

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-6052 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle (sterile water for injection) or 2, 20, 200, or 1,000 mg of T-6052/kg of body weight (Groups 1 through 5, respectively). The dose volume was 0.5 mL/kg for Groups 1 through 4 and 1.02 mL/kg for Group 5.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). A blood sample (approximately 4 mL) was collected from an auricular artery or marginal ear vein of the animals at 2-, 4-, 6-, 8-, 12-, and 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection), approximately 20 mL of blood was obtained from each animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. Approximately 48 hours post-injection, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and sent frozen to the Sponsor after termination of the in-life phase.

All five animals appeared normal throughout the study.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6052, when administered as a single intravenous injection to rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6052 and described as clear, colorless liquid. The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The purity and stability of the USP grade control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. Any unused test material will be returned to the Sponsor after completion of all testing according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any remaining vehicle may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, Michigan on October 5, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from the required temperature and humidity conditions existed, they were documented and considered to have had no adverse effect on the study outcome. Animal husbandry and housing at HWI complied with standards outlined in the "Guide for the Care and Use of Laboratory Animals".[1]

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,813 to 3,031 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg T-6052/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	*	0	0.5	1
2	T-6052	2	0.5	1
3	T-6052	20	0.5	1
4	T-6052	200	0.5	1
5	T-6052	1,000	1.02	1

* Sterile Water for Injection, USP.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Dose Preparation and Administration

The test material was diluted with Sterile Water for Injection to achieve a specific concentration for each dose level in Groups 2 through 4. The test material was administered undiluted at the 1,000 mg/kg dose level, using the bulk density of 0.98 g/mL to determine the dose volume. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (approximately 30 to 60 seconds in duration). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collection

A blood sample (approximately 4 mL) was collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all animals at 2, 4, 6, 8, 12, and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection), approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were stored at room temperature until centrifuged and separated into serum and cellular fractions. The blood samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (approximately 48-hours post-injection), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not conducted, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C. After tissue/bile collection, the animals were discarded.

Shipment of Tissues

After completion of the in-life phase the blood samples, livers, bile, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106). The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual body weights at initiation are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. All five animals appeared normal throughout the study.

Pathology

All animals survived to termination of the experimental phase and were not examined grossly when sacrificed.

DISCUSSION

The level of systemic exposure of T-6052 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 2, 20, 200, and 1,000 mg/kg. All animals appeared normal throughout the study following administration of this material.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

2-1-93
Date

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Day 0
1	0	Female	F52548	3,031
2	2	Female	F52549	2,921
3	20	Female	F52559	2,813
4	200	Female	F52566	2,912
5	1,000	Female	F52567	2,853

HWI 6329-134

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0	Female	F52548	Appeared normal	✓	✓	✓	✓	✓
2	2	Female	F52549	Appeared normal	✓	✓	✓	✓	✓
3	20	Female	F52559	Appeared normal	✓	✓	✓	✓	✓
4	200	Female	F52566	Appeared normal	✓	✓	✓	✓	✓
5	1,000	Female	F52567	Appeared normal	✓	✓	✓	✓	✓

✓ Indicates condition exists.

APPENDIX A

Protocol TP8084.PK

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WI 53707 7545

A CORNING Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

Date:

November 9, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-134

Phone [illegible]
EXPRESS MAIL [illegible]

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

HWI No.	6329-134
Test Material	T-6052
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of November 7, 1994
Experimental Termination Date	Week of November 7, 1994
Draft Report Date	Week of December 12, 1994

1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure when the test material is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6052

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

D. Storage
Room temperature

E. Reserve Samples
Reserve samples will not be required for this study.

F. Retention
Any unused test material will be discarded after issuance of the final report, unless directed otherwise by the Sponsor.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Sterile water for injection

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this USP grade material is considered to be adequate for the purposes of this study.

D. Storage
Refrigerated

E. Reserve Samples
See Section, 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.5 to 3.5 kg

(5) Number and Sex
5 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	2	1
3	20	1
4	200	1
5	1000	1

a The dose volume will be 0.5 ml/kg for Groups 1-4 and approximately 1.0 mL/kg of body weight (depending on the bulk density of the test material) for Group 5.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.

(2) Reason for Dosing Route
Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration
Single dose

(4) Dose Preparation
The test material will be diluted with sterile water for injection to achieve a specific concentration for each dose level in Groups 1-4. The test material will be administered undiluted at the 1,000 mg/kg dose level, using the bulk density to determine the dose volume. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The prepared test mixtures will be stored at room temperature until administration.

D. Observation of Animals

(1) Clinical Observations
The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from either ear via the catheterization of the auricular artery or from the marginal ear vein at 2, 4, 6, 8, 12, and 24 hours post-injection. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal at the time of necropsy (48 hours post-injection). Approximately 20 mL of blood will be collected from moribund animals during the study, also, if possible. The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain a temperature of -20°C ±10°C. The separated serum and cellular fractions will be sent frozen to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital, bled via the vena cava, and exsanguinated.

(2) Scheduled Sacrifice
At approximately 48 hours post-injection, animals surviving to termination will be anesthetized with sodium pentobarbital, bled via the vena cava, and exsanguinated. An abbreviated gross necropsy examination will not be done, however, tissues will be collected.

(a) Sample Collection
The whole liver and bile from each animal dying during the study, sacrificed in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected. The tissues will be placed on dry ice immediately after collection and then placed in a freezer set to maintain a temperature of -20°C ±10°C.

The tissues (liver, bile, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.D.(3).(c). The Sponsor is responsible for the retention and disposition of the samples.

F. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects
Gross pathology findings/gross pathology report (if applicable)

9. Location of Raw Data, Records, and Final Report

Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Sample collection records
Pathology Records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M

11-15-94
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

11-9-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

11/9/94
Date

(6329-134.protdskl)

9.1.2 Analytical protocol AMDT-111694.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-134

Study Number: AMDT-111694.1
Test Substance: FC-120 (T-6052)

Name and Address of Sponsor:
3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Author: James D. Johnson

Approved By:

James D. Johnson 10/30/95
James D. Johnson — Date
Study Director

John L. Butenhoff 10/30/95
John Butenhoff, PhD — Date
Sponsor Representative

1.0 PURPOSE

This study is performed in order to provide pharmacokinetic data for the assessment of a subsequent dermal absorption study (HWI#6329-135).

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4-liver), Sigma Chemical Company (2.1.2, 2.1.4-serum)

2.3 Number of Test and Control Samples: Liver and serum from 4 test animals and 1 control animal. Other biological tissues (kidney, bile, cellular fraction) will be available for analysis if deemed appropriate by the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity, and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), $-20 \pm 10°C$ (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-134), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver. Electrospray mass spectrometry will be performed in order to confirm the presence of specific molecules. The material being studied is a perfluorodecanesulfonic acid salt (Ammonium). This material is not expected to be biotransformed. If the material is similar to perfluorooctanesulfonic acid anion, it will be persistent in the liver. Analysis of liver for total organic fluorine will provide information as to the extent of persistence.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

4.1.6 AMDT-M-14-0,Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-120 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include averages and standard deviations from different dose groups. If necessary, simple statistical tests such as the Student's t test may be applied to determine statistical difference.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

8.6 AMDT-M-14-0,Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D Johnson
Group Leader 10/3/95
Date

Gale Van Buskirk
Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-31-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 10-31-95
Date

Quality Assurance 10-31-95
Date

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

- 4.1.1 Syringe, capable of measuring 100 µL
- 4.1.2 Eppendorf type or disposable pipets
- 4.1.3 Gloves
- 4.1.4 Plastic grinding tubes
- 4.1.5 Plastic centrifuge tubes, 15 mL
- 4.1.6 Labels
- 4.1.7 Nitrogen
- 4.1.8 Timer
- 4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
- 4.1.10 Analytical pipets: glass volumetric pipets.
- 4.1.11 Disposable plastic 3 cc syringes.
- 4.1.12 Crimp cap autovials.

4.2 **Reagents**

- 4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
- 4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
- 4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
- 4.2.4 Ethyl Acetate
- 4.2.5 Methanol
- 4.2.6 Milli-Q water
- 4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
- 4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluorooctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step 9.12 of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 **Calibration**

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 **Storage Conditions for Standards**

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 **Storage Conditions for Standards**

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in **8.3**.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 **None**

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W________	FC-95 approx 1 ppm actual ppm #W________	FC-95 approx. 5 ppm actual ppm #W________	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

Liver Extraction Process:		Date & Initials
Pipet 1 mL of Liver Solution		
Pipet 100 uL of 12 ppm Internal Standard	Std. #	
Vortex 15 sec.		
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #	
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer		
Pipet 5 mL of Ethyl Acetate		
Vortex 20-30 sec.		
Shake 20 min.		
Centrifuge 20-25 min.		
Remove a 4 mL aliquot of organic layer		
Blow down to near dryness (<0.25 mL) with N_2		
Add 1 m: of 1:1 Acetonitrile/H_2O	TN#	
Vortex 15 sec.		
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial		

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES$^-$.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES$^-$, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 Using the Autosampler

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- **9.3.3.1** Push the sample button
- **9.3.3.2** Set sample loop size = 100 uL
- **9.3.3.3** Set inject/sample = 2
- **9.3.3.4** Set Cycle time = 0
- **9.3.3.5** Name the file: Livers
- **9.3.3.6** Identify the tray used
- **9.3.3.7** Add the samples to Queue by pressing "Enter"
- **9.3.3.8** Press "Run" to start

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

Revision Number: 0 **Revision Date:** None

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Von Burkett 9-27-95
Quality Assurance Date

Software: IBM MS Word, 6.0
Affected Documents: **AMDT-EP-26,** Operation and Maintenance of the Skalar Segmented Flow Analyzer

1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps
4.1.2 Autopipets, oxford or equivalent with plastic tips
4.1.3 Polypropylene volumetric flasks, 100 mL
4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.
4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent
4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.
4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.
4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.
4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.
4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Prepare calibration standards as in section **4.3.**

8.2 Calibration

8.2.1 The standards are analyzed at the beginning of the run.

8.3 Storage Conditions for Standards

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 Start Up Procedure

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.
9.1.2 Put the fluoride electrodes in the electrode chamber.
9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.
9.1.4 Put the reagent-lines in the appropriate bottles.
9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**
9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 Starting a Run

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.
9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.
9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.
9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.
9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under **9.2.1** (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-M-1, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Serum

Method Identification Number: AMDT-M-14 **Adoption Date:** 10-3-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

James D Johnson
Group Leader 10/3/95
Date

Quality Assurance 9-27-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological fluids, particularly serum.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 100 µl. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are to be handled with plastic pipettes. A new pipette is to be used for each sample.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit serum studies, use beef serum as the matrix.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked serum curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef serum as the matrix.
8.3.2.3 Put serum blank in Dohrmann boat. Combust sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.
8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve

8.3.3.1 If beef serum is frozen, thaw at least enough to complete the standard curve analysis for the day (≈30 mL).

8.3.3.2 Pipette 100µL of beef serum into Dohrmann sample boat.

8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef serum.

8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.

8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.

8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.

8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.

8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.

8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:

9.1.1 Combustion tube temperature = 950°C.

9.1.2 Oxygen and Helium flow = 50 cc/minute.

9.1.3 Vaporization/Drying time = 240 seconds.

9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:

9.2.1 If the program is not started, start the EOX program on the PC.

9.2.2 Open the SYSTEM SETUP window.

9.2.3 Put the furnace module and the cell in the READY mode.

9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.

9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:

9.3.1 Open the SAMPLE HATCH and pipette 100µL of sample into the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.

9.3.2 Close SAMPLE HATCH.

9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-WATER program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator and/or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control

10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor | Original to: Study Director | Copies to: QAU Files

Study Title: **Single-dose Intravenous Pharmacokinetic Study of T-6052 in Rabbits**

Study Number: AMDT-111694.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/13/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-24-95

QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:
Jim Johnson
Dave Christenson

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:
Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:
Jim Johnson
Rich Youngblom

Quality Assurance Unit:
Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

Summary of Combustion Data - Liver
AMDT-111694.1, HWI 6329-134
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

	ug
Control Group	**26.3**
2.0 mg/kg dose (T6052)	**17.9**
20.0 mg/kg dose (T6052)	**17.0**
200 mg/kg dose (T6052)	**35.1**
1000 mg/kg dose (T6052)	**77.7**

FC120 PK ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
Liver Blank-1		0.355		0.109			
Liver Blank-2		0.181		0.140			
Liver Spike-1	103%	1.16		0.135			
Liver Spike-2	92%	1.34		0.105			
Liver Spike-3	84%	1.24		0.102			
F52548-1		0.328		0.106	90.3		
F52548-2		0.337	0.291	0.127	90.3	26.3	0.0
F52548-3		0.207		0.116	90.3		
F52549-1		0.179		0.101	89.3		
F52549-2		0.159	0.200	0.131	89.3	17.9	2.0
F52549-3		0.264		0.124	89.3		
F52559-1		0.277		0.107	71.9		
F52559-2		0.222	0.237	0.138	71.9	17.0	20.0
F52559-3		0.211		0.125	71.9		
F52566-1		0.298		0.143	105.1		
F52566-2		0.372	0.334	0.139	105.1	35.1	200
F52566-3		0.333		0.133	105.1		
F52567-1		0.853		0.137	89.7		
F52567-2		0.98	0.867	0.101	89.7	77.7	1000
F52567-3		0.772		0.108	89.7		
Liver Blk-1		0.133		0.112			
Liver Blk-2		0.108		0.124			
Liver Spk-1	80%	1.10		0.110			
Liver Spk-2	90%	0.980		0.139			
Liver Spk-3	92%	1.06		0.132			
Liver Spk-4	94%	2.72		0.105			
Liver Spk-5	108%	3.24		0.101			
Liver Spk-6	94%	2.43		0.117			

9.11.2 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

A-1
contains pages
A-1 through A
10-31-95
D. Christenson

HWI # 6329-134

Study:	Single-Dose Intravenous Pharmacokinetic
Protocol Number:	TP8084.PK
Test Material:	T-6052 in Rabbits (FC 120)
Matrix:	Liver
R Squared Value:	Screening
Response Factor Amount:	N/A
Analyst:	DLC
Date:	4/4/95
Method:	
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE File:	040495C

Group Dose	Sample #	Ion Count Area *	Extracted wt g	Dilution factor	Concentration µg/g **	Total mass of liver g	Total amount of FC-95 per liver mg	% of FC-95
Group 1: 0 mg/kg * Sterile Water	F52548	N.D.	1.0036	1	N.D.	90.344	N.D.	
Group 2: 2 mg /kg **	F52549	N.D.	1.0055	1	N.D.	89.284	N.D.	
Group 3: 20 mg/kg	F52559	N.D.	1.0026	1	N.D.	71.921	N.D.	
Group 4: 200 mg/kg	F52566	$	1	1		105.089		
Group 5: 1000 mg/kg	F52567	$	1.0027	1		89.664		

$ = Positive response for ion monitored.

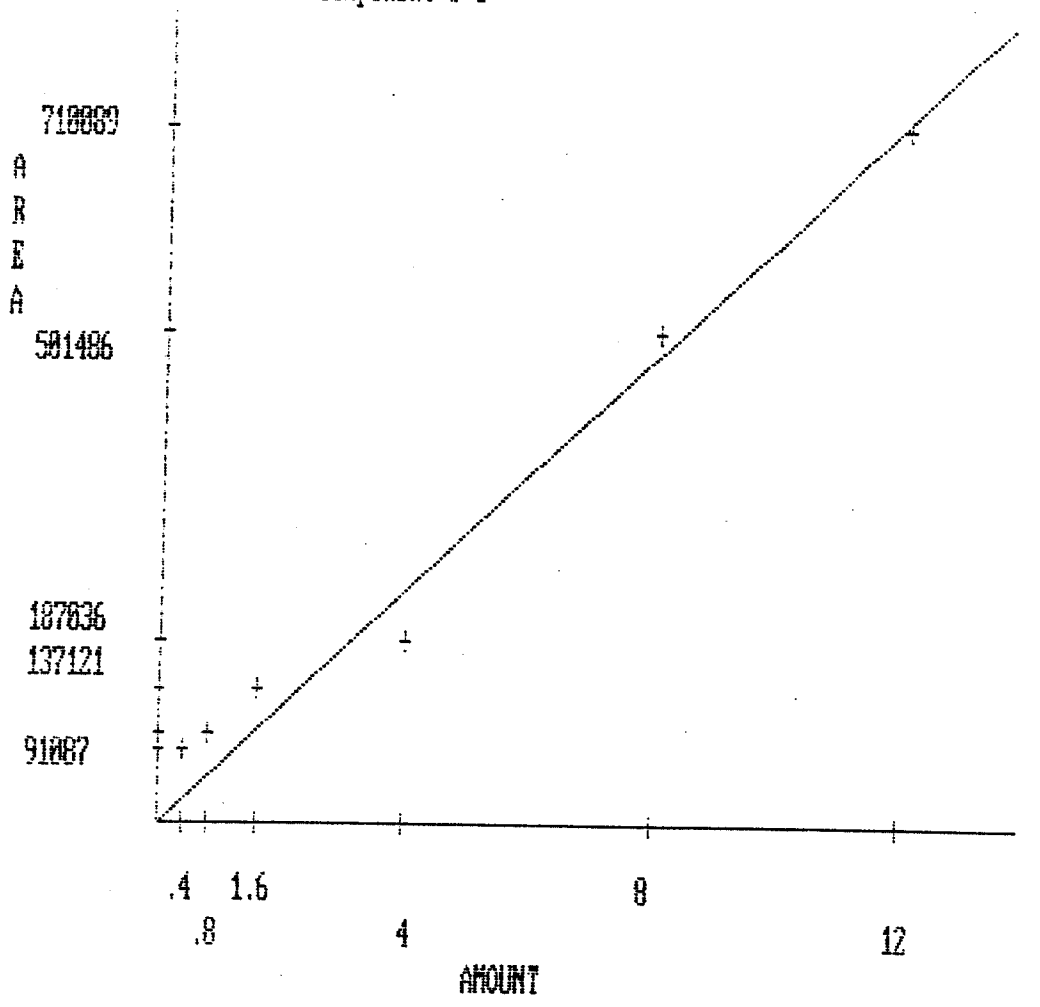
*** SIR of M+ 598 & 599**

**** The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.**

LAB BASE
FROM FILE:
040495C
HWI # 6329- ~~6328~~ 134
~~6328~~

Method DLCLIV
Sample dlcliv
Operator dlc
Run date 05-08-1995 09:34:56 Version: 1
Printed on 05-08-1995 AT 09:36:14
Straight Line Fit forced through Origin.

Component #:1



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	74739
2	0.8000	91087
3	1.6000	137121
4	4.0000	187036
5	8.0000	501486
6	12.0000	710089

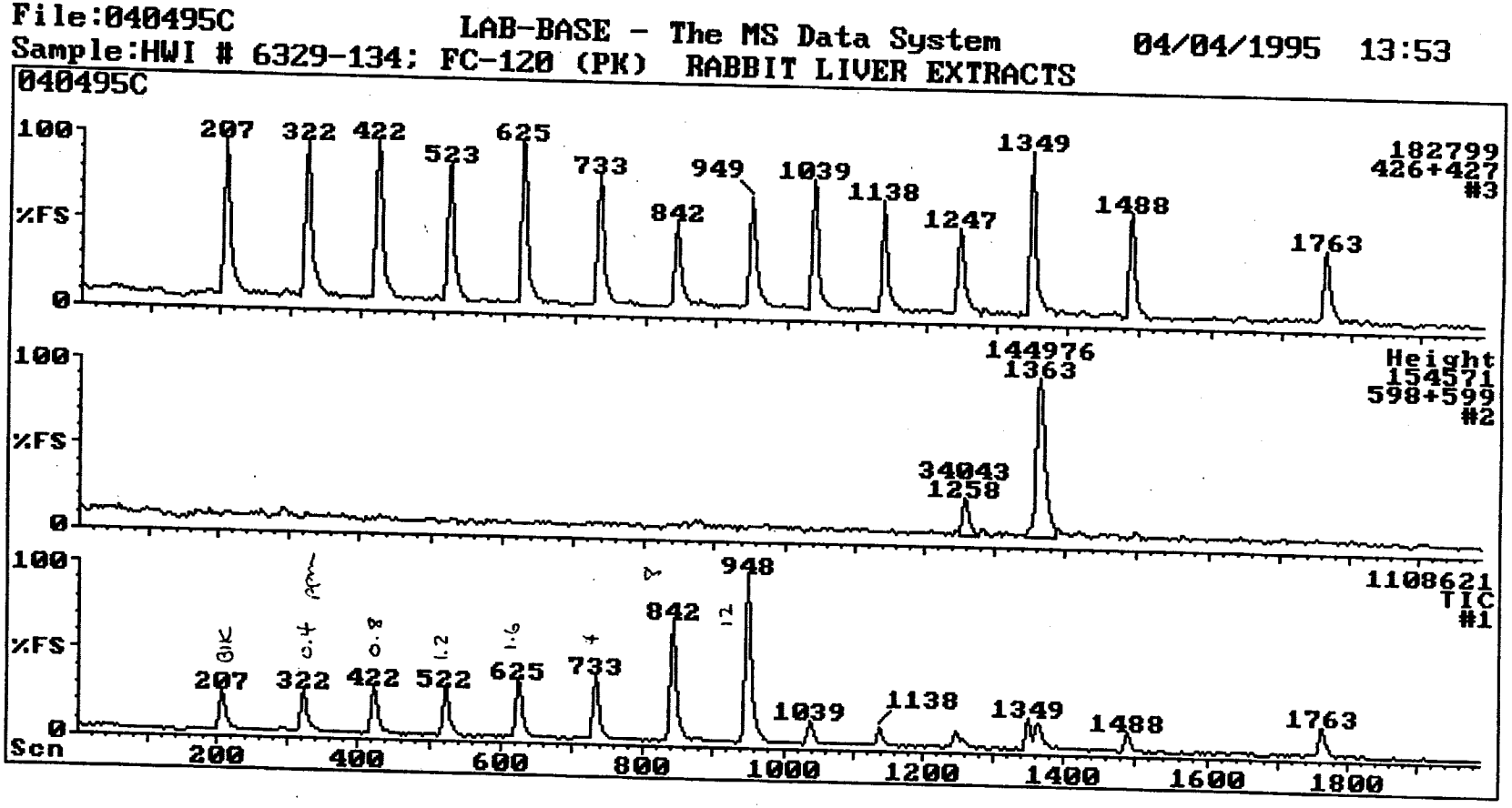
Y = SLOPE * X + INTERCEPT

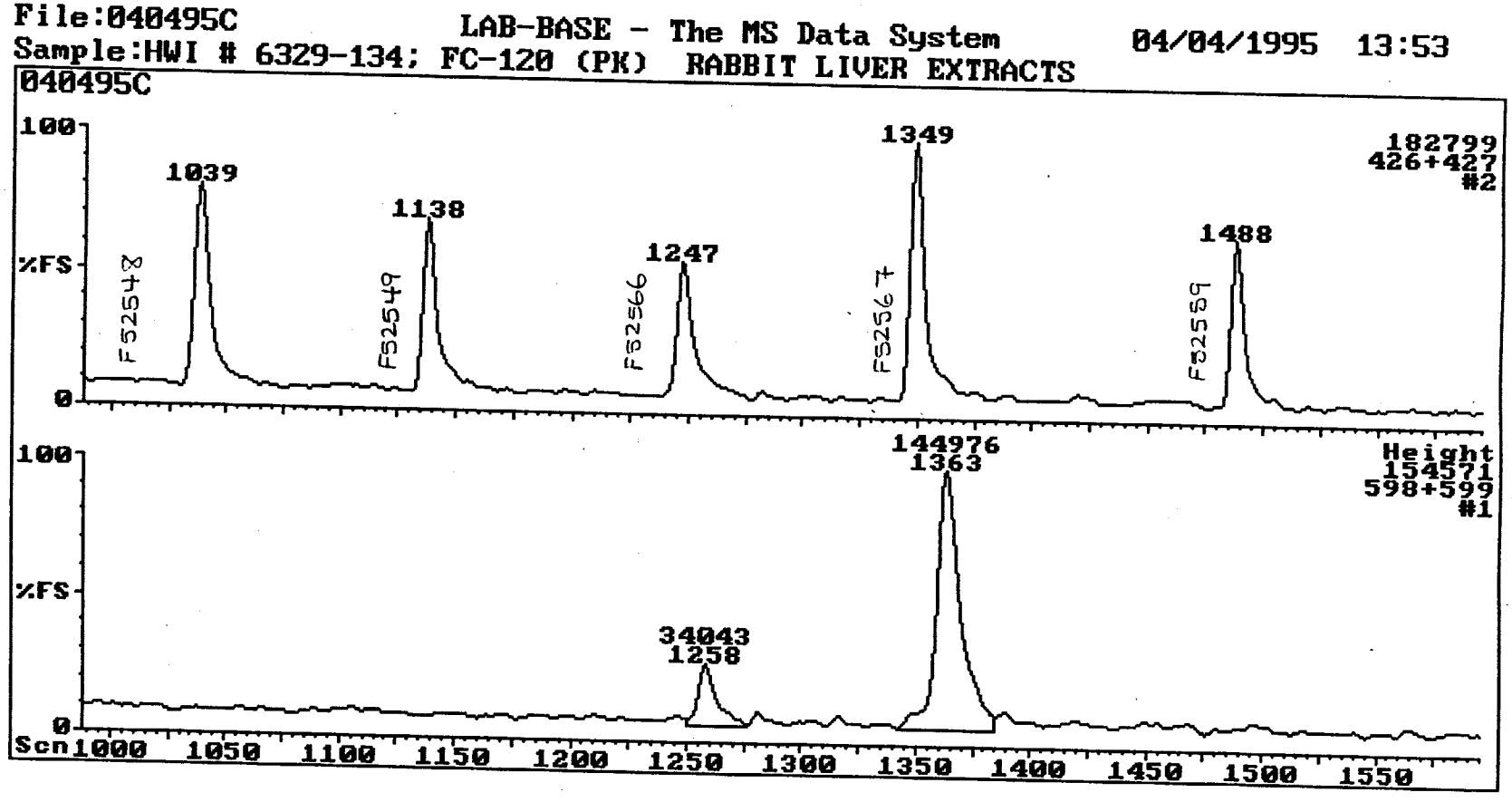
Area = 5.9831E+04 * Amount + 0.0000E+00
Amount = 1.6714E-05 * Area + 0.0000E+00
R squared = 0.9722

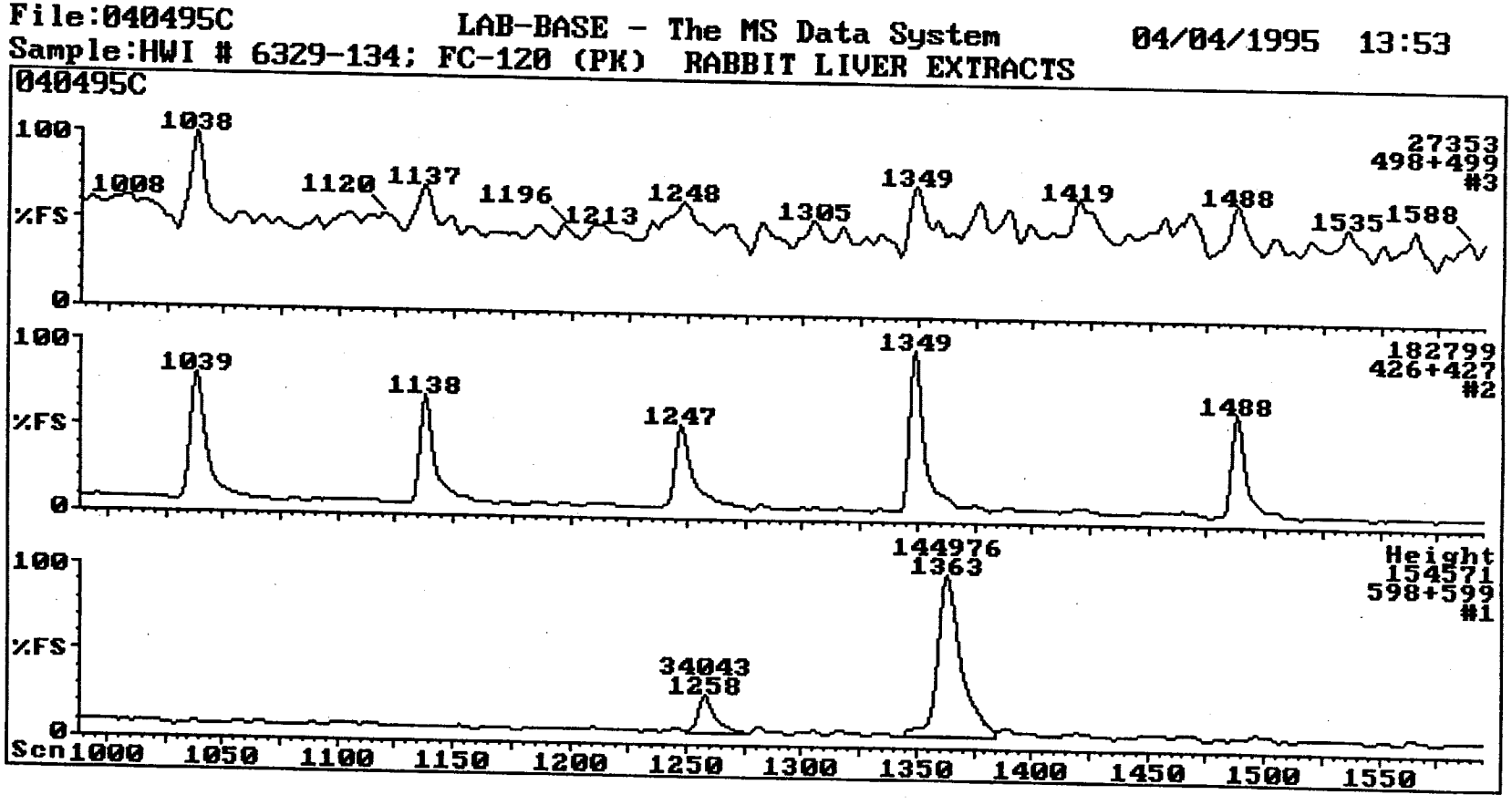
A-3

COPY

G00881




File:040495C
LAB-BASE - The MS Data System
04/04/1995 13:53
Sample:HWI # 6329-134; FC-120 (PK) RABBIT LIVER EXTRACTS
040495C
100
%FS
0
1039
1138
1247
1349
1488
182799
426+427
#2
FS2548
FS2549
FS2566
FS2567
FS2569
100
%FS
0
144976
1363
34043
1258
Height
154571
598+599
#1
Scn 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500 1550


File:040495C
LAB-BASE - The MS Data System
04/04/1995 13:53
Sample:HWI # 6329-134; FC-120 (PK) RABBIT LIVER EXTRACTS
040495C
100
%FS
0
1038
1008
1120
1137
1196
1213
1248
1305
1349
1419
1488
1535
1588
27353
498+499
#3
1039
1138
1247
1349
1488
182799
426+427
#2
144976
1363
34043
1258
Height
154571
598+599
#1
Scn
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
1500
1550

A-6

File:040495C LAB-BASE - The MS Data System 04/04/1995 13:53
Sample:HWI # 6329-134; FC-120 (PK) RABBIT LIVER EXTRACTS

9.11.3 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

RE: 6329-134 LIVER SAMPLES
AMDT 111684.1
Date of Analysis: 3-30-95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using between 0.1 and 0.2 grams of the liver. The gas is collected in 1.0 mL of 1:1 TISAB/Milli-Q water then an additional 2 mL of 1:1 TISAB/Milli-Q is added to allow for sufficient volume for Skalar analysis. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Debra D Wright

SUMMARY
OF 6329-134
LIVER SAMPLES
AMDT 111694.1

	Sample ID	Skalar Result (ppm)	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Average Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	Average Total F- per tissue (ug)
GROUP 1	F52548-1	ND	3.0	0.1057	ND	ND	90.3439	ND	ND
Dose Level : 0	F52548-3	ND	3.0	0.1160	ND		90.3439	ND	
GROUP 2	F52549-1	0.017	3.0	0.1008	0.51		89.2836	45	
Dose Level : 2 mg/kg	F52549-2	ND	3.0	0.1309	ND	ND	89.2836	ND	ND
	F52549-3	0.016	3.0	0.1239	0.39		89.2836	35	
GROUP 3	F52559-1	ND	3.0	0.1074	ND		71.9209	ND	
Dose Level : 20 mg/kg	F52559-2	0.015	3.0	0.1377	0.33	ND	71.9209	24	ND
	F52559-3	ND	3.0	0.1246	ND		71.9209	ND	
GROUP 4	F52566-1	0.019	3.0	0.1426	0.39	0.46	105.0891	41	48
Dose Level : 200 mg/kg	F52566-2	0.02	3.0	0.1393	0.52		105.0891	55	
GROUP 5	F52567-1	0.05	3.0	0.1374	1.14		89.6636	102	
Dose Level : 1000 mg/kg	F52567-2	0.04	3.0	0.1007	1.26	1.19	89.6636	113	106
	F52567-3	0.04	3.0	0.1084	1.17		89.6636	105	

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol. (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.24	83%										
2	Drift	1.50	1.27	85%										
3	Wash		0.016											
4	Std 1	0.015	0.020	131%										
5	Std 2	0.03	0.03	85%										
6	Std 3	0.06	0.06	99%										
7	Std 4	0.09	0.09	98%										
8	Std 5	0.12	0.13	105%										
9	Std 6	0.15	0.15	98%										
10	Std 7	0.30	0.29	96%										
11	Std 8	0.60	0.61	101%										
12	Std 9	1.20	1.24	103%										
13	Std 10	1.50	1.47	98%										
14	Drift	1.50	1.31	88%										
15	Wash		0.016											
16	Blk-1A		0.02		3.0	0.1087	0.61							
17	Blk-1B		ND		3.0	0.1087	ND							
18	Blk-2A		ND		3.0	0.1399	ND							
19	Blk-2B		ND		3.0	0.1399	ND							
20	Spk-1A		0.05		3.0	0.1347	1.18			0.004	63.00	0.15	0.16	105%
21	Spk-1B		0.05		3.0	0.1347	1.15			0.004	63.00	0.15	0.16	103%
22	Spk -2		0.06		3.0	0.1047	1.66			0.004	63.00	0.15	0.17	115%
23	Spk-3		0.06		3.0	0.1019	1.65			0.004	63.00	0.15	0.17	111%
24	F52548-1		ND		3.0	0.1057	ND	90.3439	ND					
25	F52548-3		ND		3.0	0.1160	ND	90.3439	ND					
26	Drift	1.50	1.28	85%										
27	Wash		0.016											
28	F52549-1		0.017		3.0	0.1008	0.51	89.2836	45					
29	F52549-2		ND		3.0	0.1309	ND	89.2836	ND					
30	F52549-3		0.016		3.0	0.1239	0.38	89.2836	34					
31	F52559-1		ND		3.0	0.1074	ND	71.9209	ND					
32	F52559-2		0.015		3.0	0.1377	0.33	71.9209	24					
33	F52559-3		ND		3.0	0.1246	ND	71.9209	ND					
34	F52566-1		0.019		3.0	0.1426	0.39	105.0891	41					
35	F52566-2		0.02		3.0	0.1393	0.52	105.0891	55					
36	F52567-1		0.05		3.0	0.1374	1.14	89.6636	102					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	Total Tissue Wt. (grams)	Total F- per tissue (ug)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug Fe)	% Recovery
37	F52567-2		0.04		3.0	0.1007	1.26	89.6636	113					
38	Drift	1.50	1.26	84%										
39	Wash		0.016											
40	F52567-3		0.04		3.0	0.1084	1.17	89.6636	105					
41	Drift	1.50	1.28	85%										
42	Wash		0.016											

1995-03-30 13:29 **OutPut of : 950330B1**

Software : version 6.1 c1990,93

Operator : ddw

Date of the Analysis : 1995-03-30 11:18

Analysis File Name : C:\SKALAR\DATA\950330B1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

```
          Ö x - c1 ¢      x  = corrected value of the sample
          ° ááááá °      c1 = corrected value of the concentration 1
Result = 10â   s    ì      s  = Slope of the electrode
```

a2 = -0.00000
a1 = 0.00085
a0 = -1.13248

Fluoride L
Calibration order = 2

Correlation : r = 0.99665

Result = a2 * x» + a1 * x + a0

a2 = 0.00000
a1 = 0.00031
a0 = 0.01615

```
Sampler     Type          : SA1000
            Number        : 1
            Sample Time   : 50  sec.
            Wash   Time   : 120 sec.
            Air    Time   : 1   sec.
            Take up       : Single
            sPecial       : None
            needle Height : 70  mm.

Diluter     needle Height   : 80   mm
            dilution Factor : 10
            dilution Volume : 2.5  ml.
            Resample        : 1
            Dilution runs   : 1

            User file :          . TXT
            Reproces  : No
```

1995-03-30 13:29 OutPut of : 950330B1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

Fluoride 1.5
Fluoride L

PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F Cor.	Valu	Time
wt	iw	Initial Wash	1	1.000	3	0.074	0	128	65
					4	0.0161	0	0	0
1	t	Tracer	1	1.000	3	1.242	2167	2313	212
					4	0.8874	2167	0	0
2	d	Drift	1	1.000	3	1.274	2207	2370	388
					4	0.9072	2207	0	0
3	w	Wash	1	1.000	3	0.074	0	181	574
					4	0.0161	0	0	0
4	s1	Standard 1	1	1.000	3	0.075	11	192	747
					4	0.0196	11	0	0
5	s2	Standard 2	1	1.000	3	0.078	30	212	911
					4	0.0255	30	0	0
6	s3	Standard 3	1	1.000	3	0.096	137	320	1086
					4	0.0594	137	0	0
7	s4	Standard 4	1	1.000	3	0.113	226	410	1264
					4	0.0883	226	0	0
8	s5	Standard 5	1	1.000	3	0.138	338	524	1437
					4	0.1257	338	0	0
9	s6	Standard 6	1	1.000	3	0.153	399	586	1612
					4	0.1465	399	0	0
10	s7	Standard 7	1	1.000	3	0.289	797	992	1787
					4	0.2901	797	0	0
11	s8	Standard 8	1	1.000	3	0.605	1362	1568	1963
					4	0.5171	1362	0	0
12	s9	Standard 9	1	1.000	3	1.239	2163	2386	2137
					4	0.8854	2163	0	0
13	s10	Standard 10	1	1.000	3	1.466	2460	2694	2312
					4	1.0359	2460	0	0
14	d	Drift	1	1.000	3	1.313	2256	2440	2487
					4	0.9317	2256	0	0
15	w	Wash	1	1.000	3	0.074	0	184	2668
					4	0.0161	0	0	0

Fluoride 1.5
Fluoride L

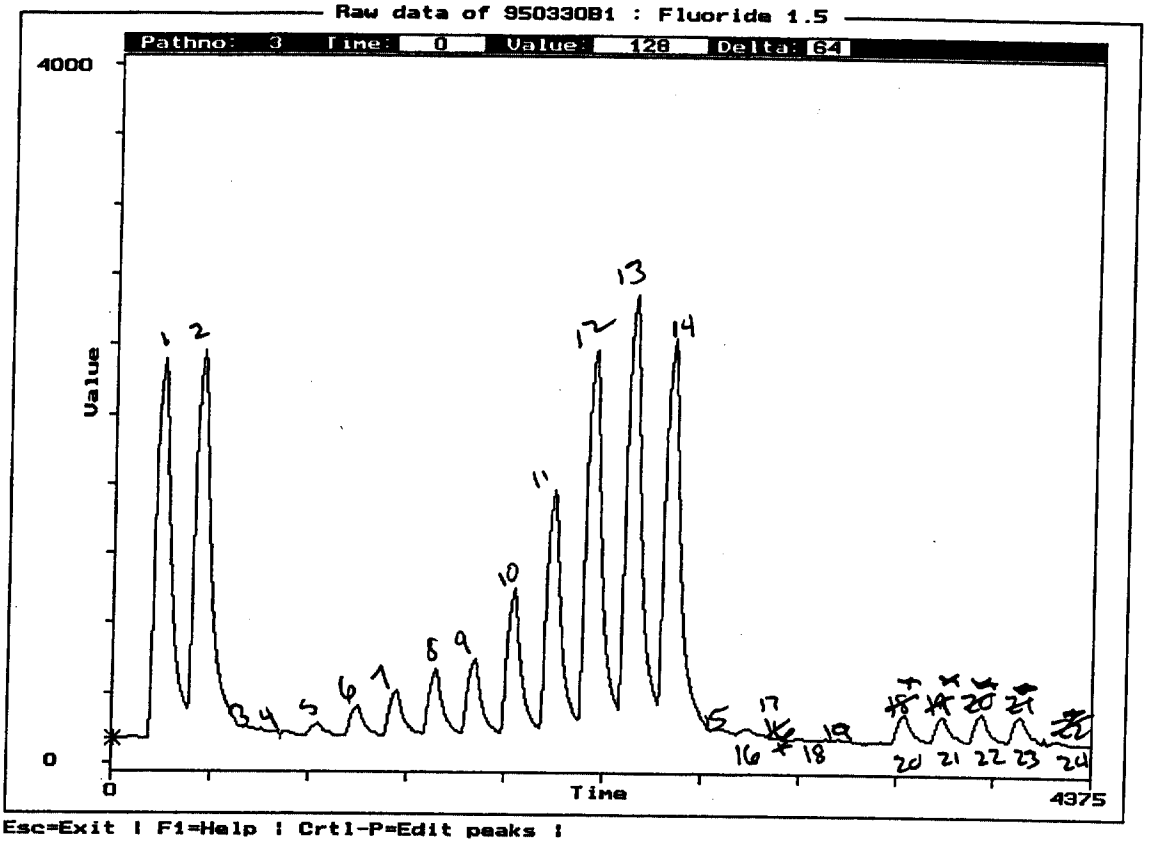
PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F Cor.	Valu	Time
16	u	BLK 1-A	1	1.000	3	0.077	19	200	2836
					4	0.0220	19	0	0
17	u	BLK 1-B	1	1.000	3	Absen A	-39	138	3012
					4	0.0041	-39	0	0
18	u	BLK 2-A	1	1.000	3	too l >	-27	148	3242
					4	0.0078	-27	0	0
19	u	BLK 2-B	1	1.000	3	0.068	-43	128	3360
					4	0.0029	-43	0	0
20	u	SPK 1-A	1	1.000	3	0.092	117	288	3536
					4	0.0530	117	0	0
21	u	SPK 1-B	1	1.000	3	0.092	113	280	3714
					4	0.0517	113	0	0
22	u	SPK 2	1	1.000	3	0.095	133	298	3888
					4	0.0581	133	0	0
23	u	SPK 3	1	1.000	3	0.094	126	288	4059
					4	0.0559	126	0	0
24	u	F52548-1	1	1.000	3	0.072	-13	145	4236
					4	0.0121	-13	0	0
25	u	F52548-3	1	1.000	3	Absen A	-23	132	4412
					4	0.0090	-23	0	0
26	d	Drift	1	1.000	3	1.282	2216	2368	4588
					4	0.9117	2216	0	0
27	w	Wash	1	1.000	3	0.074	0	149	4771
					4	0.0161	0	0	0
28	u	F52549-1	1	1.000	3	0.074	3	152	4904
					4	0.0171	3	0	0
29	u	F52549-2	1	1.000	3	Absen A	-22	128	5113
					4	0.0094	-22	0	0
30	u	F52549-3	1	1.000	3	0.073	-2	148	5285
					4	0.0155	-2	0	0
31	u	F52559-1	1	1.000	3	0.073	-7	144	5461
					4	0.0140	-7	0	0

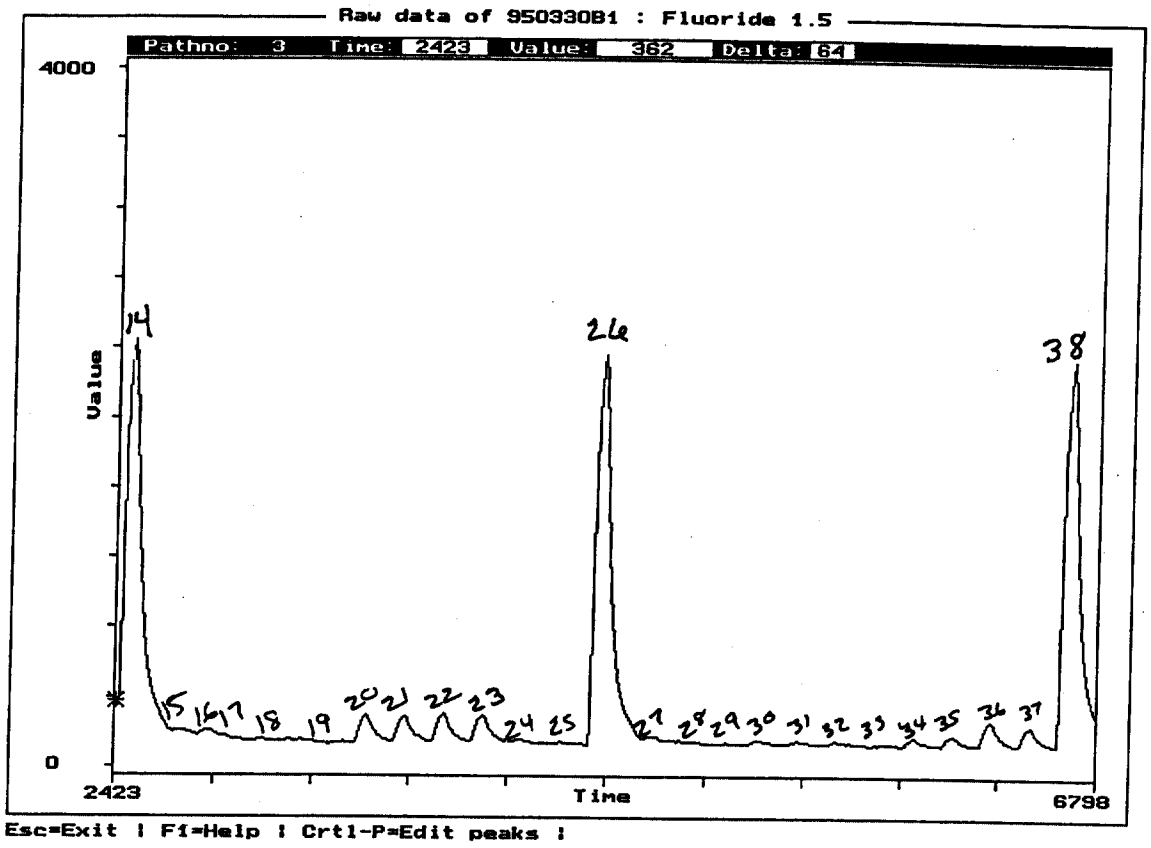
Fluoride 1.5
Fluoride L

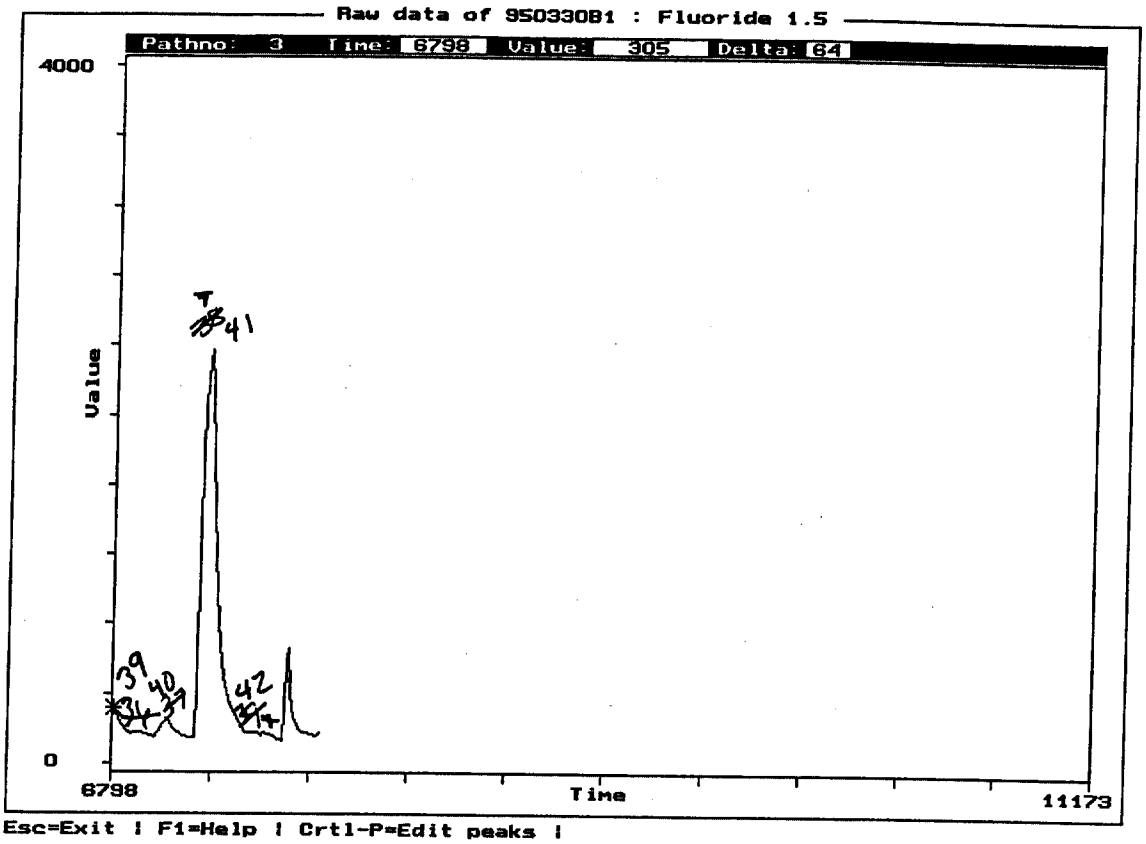
PPM
PPM

Pos	Typ	Ident	Dil	Weight	Ch	Result	F	Cor.	Valu	Time
32	u	F52559-2	1	1.000	3	0.073		-3	148	5606
					4	0.0152		-3	0	0
33	u	F52559-3	1	1.000	3	Absen	A	-31	120	5813
					4	0.0066		-31	0	0
34	u	F52566-1	1	1.000	3	0.075		8	160	5982
					4	0.0186		8	0	0
35	u	F52566-2	1	1.000	3	0.078		26	178	6163
					4	0.0242		26	0	0
36	u	F52567-1	1	1.000	3	0.092		114	266	6338
					4	0.0520		114	0	0
37	u	F52567-2	1	1.000	3	0.087		83	236	6513
					4	0.0422		83	0	0
38	d	Drift	1	1.000	3	1.262		2192	2346	6689
					4	0.8998		2192	0	0
39	w	Wash	1	1.000	3	0.074		0	154	6871
					4	0.0161		0	0	0
40	u	F52567-3	1	1.000	3	0.087		83	236	7038
					4	0.0422		83	0	0
41	d	Drift	1	1.000	3	1.282		2217	2368	7213
					4	0.9122		2217	0	0
42	w	Wash	1	1.000	3	0.074		0	150	7450
					4	0.0161		0	0	0
wt	rw	RunOut Wash	1	1.000	3	0.074		0	152	7688
					4	0.0161		0	0	0

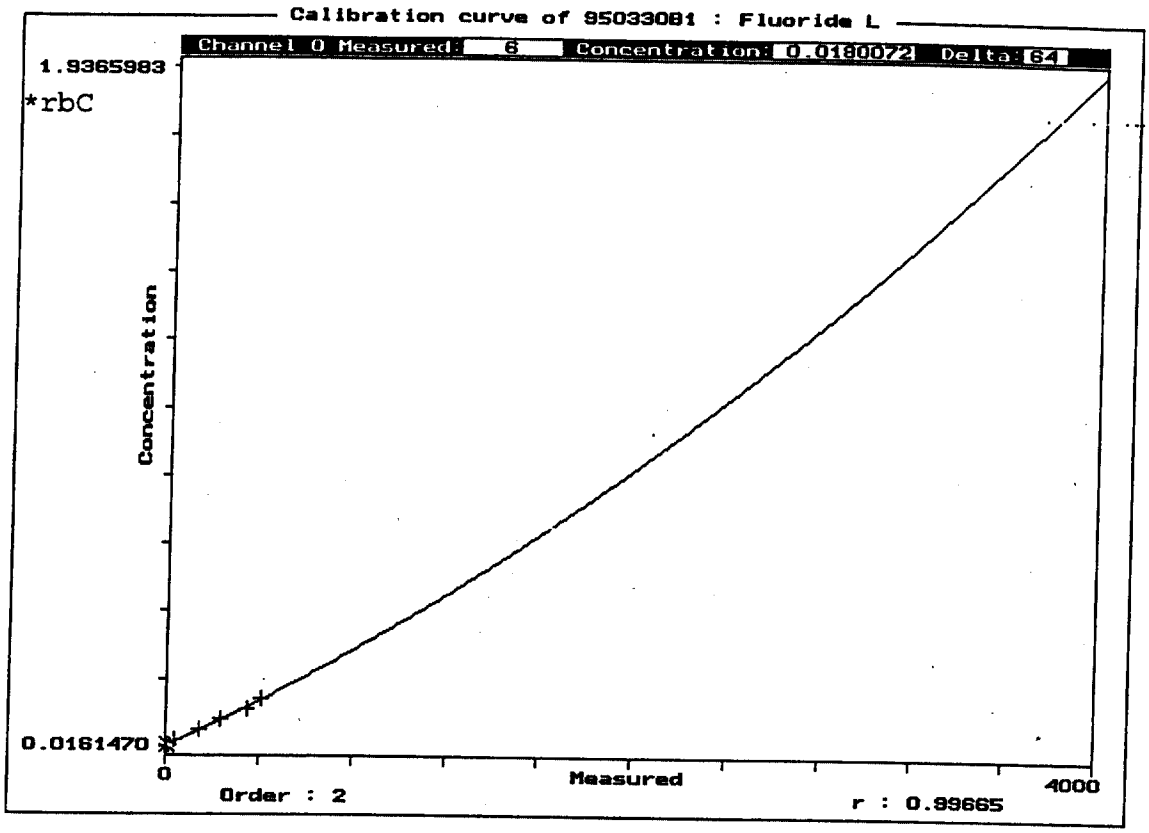

Raw data of 950330B1 : Fluoride 1.5
Pathno: 3 Time: 0 Value: 126 Delta: 64
4000
Value
0
0
Time
4375
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !

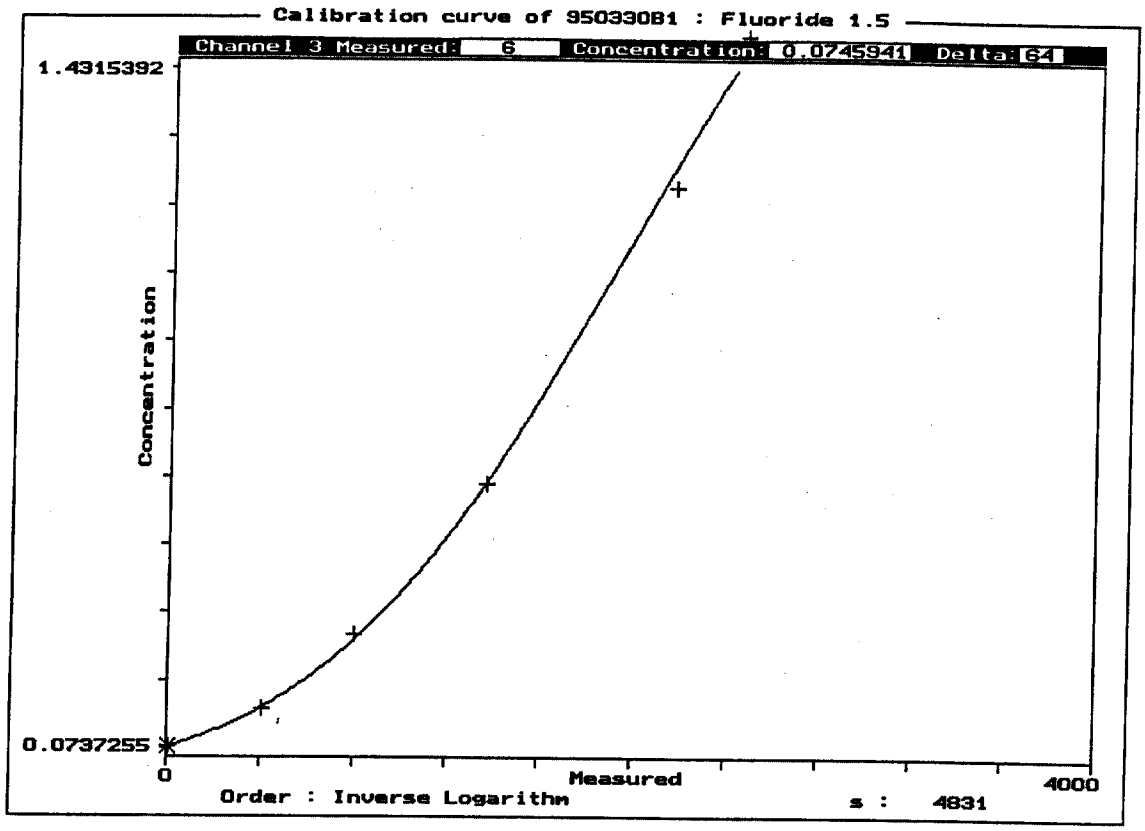
* DDW TE 7/18/95


Raw data of 95033081 : Fluoride 1.5
Pathno: 3 Time: 2423 Value: 362 Delta: 64
4000
0
Value
2423
Time
6798
14
26
38
15 16 17 18 19 20 21 22 23 24 25 27 28 29 30 31 32 33 34 35 36 37
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !


Raw data of 950330B1 : Fluoride 1.5
Pathno: 3 Time: 6798 Value: 305 Delta: 64
4000
Value
0
6798
Time
11173
41
39
40
42
Esc=Exit ! F1=Help ! Crtl-P=Edit peaks !

* DAW TE 7/18/95


Calibration curve of 95033081 : Fluoride L
Channel 0 Measured: 6 Concentration: 0.0180072 Delta: 64
1.9365983
*rbC
Concentration
0.0161470
0
Measured
4000
Order : 2
r : 0.99665


Calibration curve of 95033081 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0745941
Delta: 64
1.4315392
0.0737255
Concentration
0
Measured
4000
Order : Inverse Logarithm
s : 4831

9.11.4 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

HWI 6329-134
AMDT 111694.1
Dohrmann Serum Analysis
Analysis Dates: 07/31/95 - 08/2/95

All serum samples were thermally extracted by a modified Dohrmann DX2000 Organic Halide Analyzer and collected in a 1:1 milli Q water and TISAB solution. The samples were measured on an Orion EA940 expandable ion analyzer. The Dohrmann was calibrated using 34ppm, 40ppm, 62ppm, 100ppm, 124ppm, 250ppm, and 500ppm FC-95 standards. The Orion was calibrated by direct measurement with no blank correction using 0.05ppm, 0.1ppm, 0.5ppm, 1.0ppm and 1.5ppm F^- standards. The slope, intercept, and correlation were recorded in the appropriate logbook.

A summary table is included, showing the ppm F^- in each sample (see page 2). The summary table also shows the actual Orion readings. An initial calibration curve with standard deviation, %RSD, R^2 value and equation of the line is on pages 3 and 4.

Pages 5 and 6 show the excel spreadsheet that was generated when the samples were being analyzed.

The Dohrmann FC95 calibration curve was not used to generate the data.

Deann K. Plummer
8/14/95

FC120 PK

HWI 6329-134

Fluoride concentration in rabbit serum (ppm F-)

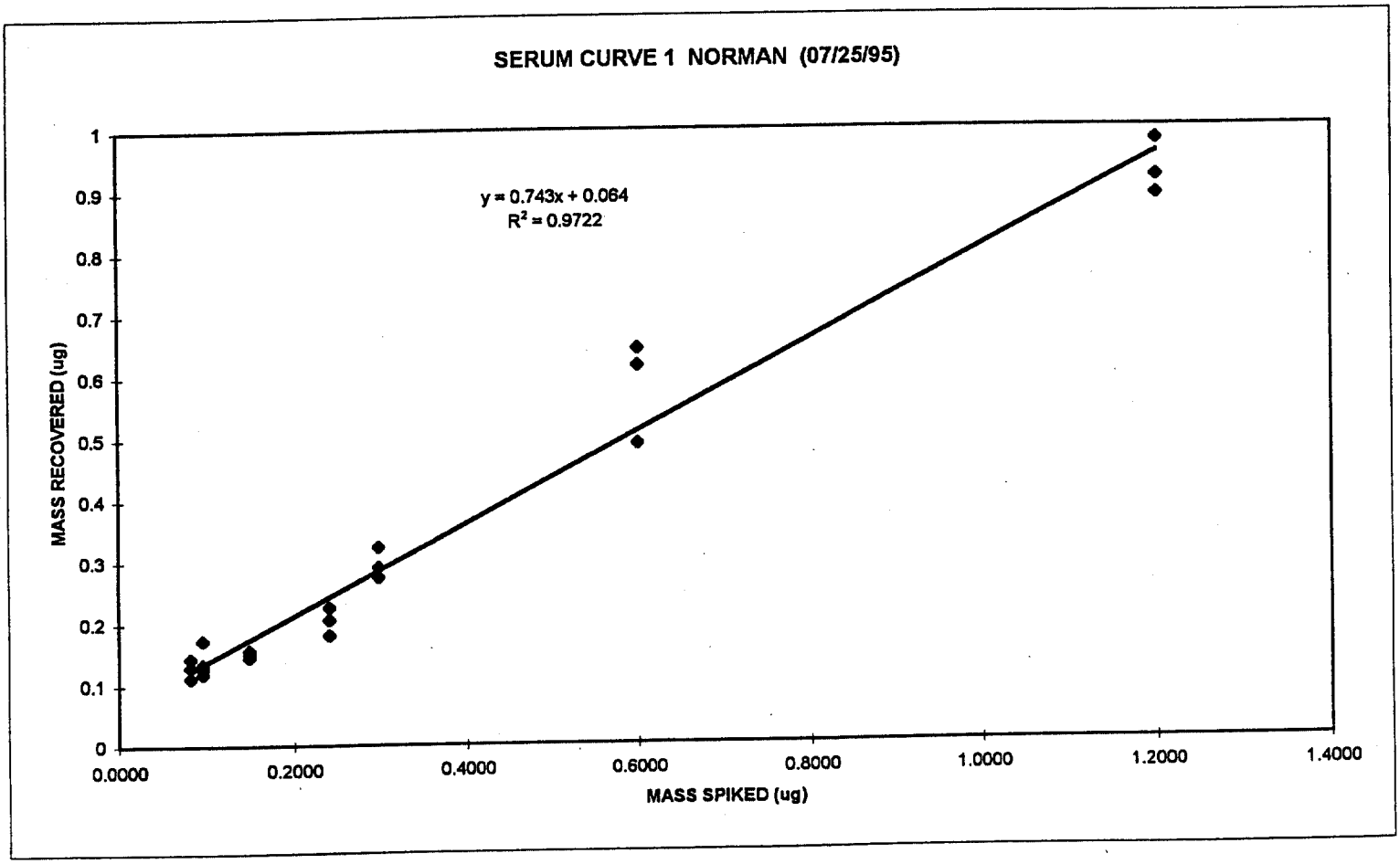
		Sample	2 hour	4 hour	6 hour	8 hour	12 hour	24 hour	48 hour
Dosage:	0 mg/kg	F52548	0.453	0.359	0.334	0.411	0.383	1.70	0.678
	2 mg/kg	F52549	0.372	0.329	0.322	0.384	0.353	0.68	0.642
	20 mg/kg	F52559	0.357	0.283	0.532	0.291	0.290	0.584	0.594
	200 mg/kg	F52566	0.400	0.282	0.539	0.436	0.296	0.564	0.718
	1000 mg/kg	F52567	0.402	0.367	0.427	0.519	0.327	0.684	0.560

Actual Orion Reading (ppm F-)

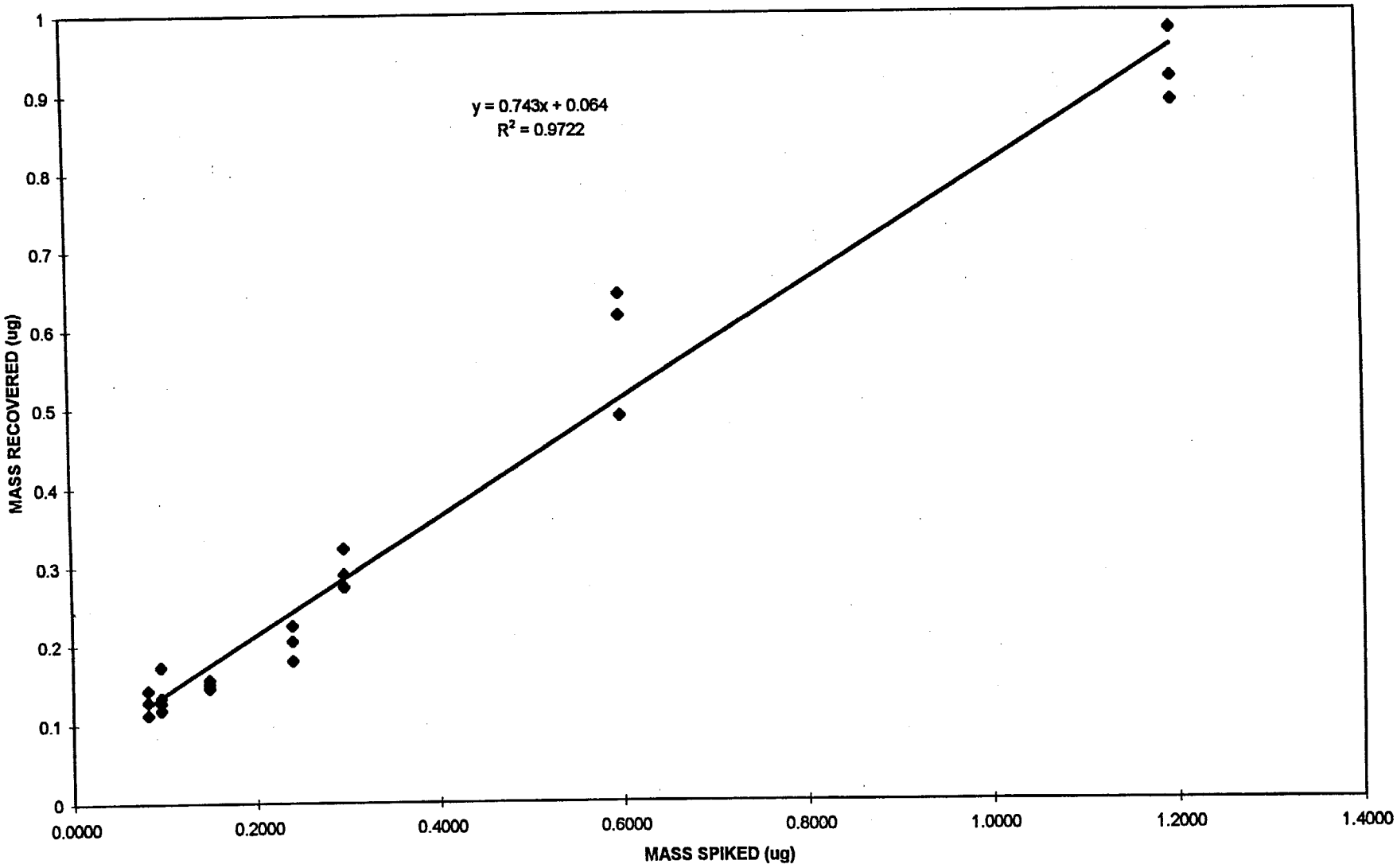
		Sample	2 hour	4 hour	6 hour	8 hour	12 hour	24 hour	48 hour
Dosage:	0 mg/kg	F52548	0.0226	0.0180	0.0167	0.0206	0.0191	0.0848	0.0339
	2 mg/kg	F52549	0.0186	0.0164	0.0161	0.0192	0.0177	0.0340	0.0321
	20 mg/kg	F52559	0.0179	0.0142	0.0266	0.0145	0.0145	0.0292	0.0297
	200 mg/kg	F52566	0.200	0.0141	0.0270	0.0218	0.0148	0.0282	0.0359
	1000 mg/kg	F52567	0.0201	0.0183	0.0214	0.0260	0.0164	0.0342	0.0280

NORMAN SERUM CURVE 1

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
34-ppm-1	0.07175	0.1	2.0	0.004	34	176%	1.4350	0.0817	0.1435	STDEV:	0.015629
34-ppm-2	0.05614	0.1	2.0	0.004	34	138%	1.1228	0.0817	0.11228	%RSD:	12
34-ppm-3	0.06462	0.1	2.0	0.004	34	158%	1.2924	0.0817	0.12924		
40-ppm-1	0.08668	0.1	2.0	0.004	40	180%	1.7336	0.0961	0.17336	STDEV:	0.024057
40-ppm-2	0.06728	0.1	2.0	0.004	40	140%	1.3456	0.0961	0.13456	%RSD:	17
40-ppm-3	0.05939	0.1	2.0	0.004	40	124%	1.1878	0.0961	0.11878		
40-ppm-4	0.06385	0.1	2.0	0.004	40	133%	1.2770	0.0961	0.1277		
62-ppm-1	0.07291	0.1	2.0	0.004	62	98%	1.4582	0.1489	0.14582	STDEV:	0.005495
62-ppm-2	0.0753	0.1	2.0	0.004	62	101%	1.5060	0.1489	0.1506	%RSD:	3.6
62-ppm-3	0.07839	0.1	2.0	0.004	62	105%	1.5678	0.1489	0.15678		
100-ppm-1	0.0902	0.1	2.0	0.004	100	75%	1.8040	0.2402	0.1804	STDEV:	0.022443
100-ppm-2	0.1026	0.1	2.0	0.004	100	85%	2.0520	0.2402	0.2052	%RSD:	11
100-ppm-3	0.1126	0.1	2.0	0.004	100	94%	2.2520	0.2402	0.2252		
124-ppm-1	0.1371	0.1	2.0	0.004	124	92%	2.7420	0.2978	0.2742	STDEV:	0.025096
124-ppm-2	0.1451	0.1	2.0	0.004	124	97%	2.9020	0.2978	0.2902	%RSD:	8.5
124-ppm-3	0.1617	0.1	2.0	0.004	124	109%	3.2340	0.2978	0.3234		
250-ppm-1	0.3217	0.1	2.0	0.004	250	107%	6.4340	0.6004	0.6434	STDEV:	0.082072
250-ppm-2	0.2447	0.1	2.0	0.004	250	82%	4.8940	0.6004	0.4894	%RSD:	14
250-ppm-3	0.3078	0.1	2.0	0.004	250	103%	6.1560	0.6004	0.6156		
500-ppm-1	0.4438	0.1	2.0	0.004	500	74%	8.8760	1.2008	0.8876	STDEV:	0.045915
500-ppm-2	0.4584	0.1	2.0	0.004	500	76%	9.1680	1.2008	0.9168	%RSD:	5.0
500-ppm-3	0.4888	0.1	2.0	0.004	500	81%	9.7760	1.2008	0.9776		



SERUM CURVE 1 NORMAN (07/25/95)



98 Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	onc. FC9 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
BLANK-1	0.0830	0.1	2.0				1.66		0.166
BLANK-2	0.0314	0.1	2.0				0.627		0.0627
SPIKE 62-1	0.0635	0.1	2.0	0.004	62	85%	1.27	0.15	0.127
SPIKE 62-2	0.0644	0.1	2.0	0.004	62	86%	1.29	0.15	0.129
SPIKE250-1	0.157	0.1	2.0	0.004	250	52%	3.15	0.60	0.315
SPIKE250-2	0.239	0.1	2.0	0.004	250	80%	4.78	0.60	0.478
SPIKE250-3	0.240	0.1	2.0	0.004	250	80%	4.80	0.60	0.480
F52548-2HR	0.0226	0.1	2.0				0.453		0.0453
F52549-2HR	0.0186	0.1	2.0				0.372		0.0372
F52559-2HR	0.0179	0.1	2.0				0.357		0.0357
F52566-2HR	0.0200	0.1	2.0				0.400		0.0400
F52567-2HR	0.0201	0.1	2.0				0.402		0.0402
62PPM-3	0.0560	0.1	2.0	0.004	62	75%	1.12	0.15	0.112
62PPM-4	0.0834	0.1	2.0	0.004	62	112%	1.67	0.15	0.167
250PPM-3	0.215	0.1	2.0	0.004	250	72%	4.29	0.60	0.429
250PPM-4	0.240	0.1	2.0	0.004	250	80%	4.80	0.60	0.480
BLANK-1	0.0722	0.1	2.0				1.44		0.144
BLANK-2	0.0386	0.1	2.0				0.772		0.0772
BLANK-3	0.0317	0.1	2.0				0.633		0.0633
BLANK-4	0.0340	0.1	2.0				0.680		0.0680
BLANK-5	0.0247	0.1	2.0				0.493		0.0493
SPIKE 62-1	0.0658	0.1	2.0	0.004	62	88%	1.32	0.15	0.132
SPIKE 62-2	0.0802	0.1	2.0	0.004	62	108%	1.60	0.15	0.160
SPIKE 250-1	0.200	0.1	2.0	0.004	250	67%	4.00	0.60	0.400
SPIKE 250-2	0.204	0.1	2.0	0.004	250	68%	4.07	0.60	0.407
SPIKE 250-3	0.253	0.1	2.0	0.004	250	84%	5.07	0.60	0.507
SPIKE 250-4	0.185	0.1	2.0	0.004	250	61%	3.69	0.60	0.369
BLANK	0.0796	0.1	2.0				1.59		0.159
BLANK	0.0237	0.1	2.0				0.475		0.0475
F52548-4HR	0.0180	0.1	2.0				0.359		0.0359
F52549-4HR	0.0164	0.1	2.0				0.329		0.0329
F52559-4HR	0.0142	0.1	2.0				0.283		0.0283
F52566-4HR	0.0141	0.1	2.0				0.282		0.0282
F52567-4HR	0.0183	0.1	2.0				0.367		0.0367
F52548-6HR	0.0167	0.1	2.0				0.334		0.0334
F52549-6HR	0.0161	0.1	2.0				0.322		0.0322
F52559-6HR	0.0266	0.1	2.0				0.532		0.0532
62-PPM-1	0.0542	0.1	2.0	0.004	62	73%	1.08	0.15	0.108
62-PPM-2	0.0722	0.1	2.0	0.004	62	97%	1.44	0.15	0.144
250-PPM-1	0.101	0.1	2.0	0.004	250	33%	2.01	0.60	0.201
250-PPM-2	0.159	0.1	2.0	0.004	250	53%	3.17	0.60	0.317
250-PPM-3	0.251	0.1	2.0	0.004	250	84%	5.03	0.60	0.503
BLANK	0.786	0.1	2.0				15.7		1.57
F52566-6HR	0.0270	0.1	2.0				0.539		0.0539
F52567-6HR	0.0214	0.1	2.0				0.427		0.0427
F52548-8HR	0.0206	0.1	2.0				0.411		0.0411
F52549-8HR	0.0192	0.1	2.0				0.384		0.0384
F52559-8HR	0.0145	0.1	2.0				0.291		0.0291

98 Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	conc. FC9 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
F52566-8HR	0.0218	0.1	2.0				0.436		0.0436
F52567-8HR	0.0260	0.1	2.0				0.519		0.0519
F52548-12HR	0.0191	0.1	2.0				0.383		0.0383
F52549-12HR	0.0177	0.1	2.0				0.353		0.0353
F52559-12HR	0.0145	0.1	2.0				0.290		0.0290
F52566-12HR	0.0148	0.1	2.0				0.296		0.0296
F52567-12HR	0.0164	0.1	2.0				0.327		0.0327
62-PPM-1	0.0744	0.1	2.0	0.004	62	100%	1.49	0.15	0.149
62-PPM-2	0.0915	0.1	2.0	0.004	62	123%	1.83	0.15	0.183
250-PPM-1	0.284	0.1	2.0	0.004	250	95%	5.68	0.60	0.568
250-PPM-2	0.339	0.1	2.0	0.004	250	113%	6.79	0.60	0.679
BLANK	0.0485	0.1	2.0				0.970		0.0970
BLANK	0.0471	0.1	2.0				0.942		0.0942
BLANK	0.0295	0.1	2.0				0.590		0.0590
BLANK	0.0304	0.1	2.0				0.607		0.0607
BLANK	0.0301	0.1	2.0				0.602		0.0602
62-PPM-1	0.0583	0.1	2.0	0.004	62	78%	1.17	0.15	0.117
62-PPM-2	0.0803	0.1	2.0	0.004	62	108%	1.61	0.15	0.161
250-PPM-1	0.186	0.1	2.0	0.004	250	62%	3.72	0.60	0.372
250-PPM-2	0.175	0.1	2.0	0.004	250	58%	3.50	0.60	0.350
250-PPM-3	0.201	0.1	2.0	0.004	250	67%	4.02	0.60	0.402
250-PPM-4	0.247	0.1	2.0	0.004	250	82%	4.94	0.60	0.494
250-PPM-5	0.228	0.1	2.0	0.004	250	76%	4.56	0.60	0.456
BLANK	0.105	0.1	2.0				2.10		0.210
BLANK	0.0205	0.1	2.0				0.410		0.0410
F52548-24HR	0.0848	0.1	2.0				1.70		0.170
F52549-24HR	0.0340	0.1	2.0				0.680		0.0680
F52559-24HR	0.0292	0.1	2.0				0.584		0.0584
F52566-24HR	0.0282	0.1	2.0				0.564		0.0564
F52567-24HR	0.0342	0.1	2.0				0.684		0.0684
F52548-48HR	0.0339	0.1	2.0				0.678		0.0678
F52549-48HR	0.0321	0.1	2.0				0.642		0.0642
F52559-48HR	0.0297	0.1	2.0				0.594		0.0594
F52566-48HR	0.0359	0.1	2.0				0.718		0.0718
F52567-48HR	0.0280	0.1	2.0				0.560		0.0560
BLANK	0.0613	0.1	2.0				1.23		0.123
BLANK	0.0396	0.1	2.0				0.792		0.0792
BLANK	0.0383	0.1	2.0				0.766		0.0766
BLANK	0.0412	0.1	2.0				0.824		0.0824
BLANK	0.0319	0.1	2.0				0.638		0.0638
62-PPM-1	0.0786	0.1	2.0	0.004	62	106%	1.57	0.15	0.157
62-PPM-2	0.0934	0.1	2.0	0.004	62	125%	1.87	0.15	0.187
250-PPM-1	0.304	0.1	2.0	0.004	250	101%	6.08	0.60	0.608
250-PPM-2	0.269	0.1	2.0	0.004	250	90%	5.38	0.60	0.538

9.11.5 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

Skalar Data

RE: 6329-134 SERUM SAMPLES
AMDT 111694.1
Date of Analysis: 8/8/95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using 0.10 mL of the serum. The gas is collected in 2.0 mL of 1:1 TISAB/Milli-Q water. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Deborah D Wright

Stalar Rat

SUMMARY OF 6329-134
SERUM SAMPLES
AMDT 111694.1

	Sample ID	Fluoride in Sample (ppm) 2 hr	Fluoride in Sample (ppm) 4 hr	Fluoride in Sample (ppm) 6 hr	Fluoride in Sample (ppm) 8 hr	Fluoride in Sample (ppm) 12 hr	Fluoride in Sample (ppm) 24 hr	Fluoride in Sample (ppm) 48 hr
GROUP 1 Dose Level : 0	F52548	0.79	0.46	0.43	0.77	0.44	2.26	0.88
GROUP 2 Dose Level : 2 mg/kg	F52549	0.52	0.55	0.40	0.57	0.39	0.91	0.83
GROUP 3 Dose Level : 20 mg/kg	F52559	0.52	0.39	0.75	0.35	ND	0.87	0.76
GROUP 4 Dose Level : 200 mg/kg	F52566	0.72	0.34	0.86	0.56	0.31	0.77	0.94
GROUP 5 Dose Level : 1000 mg/kg	F52567	0.62	0.51	0.65	0.66	0.42	0.89	0.72

600310

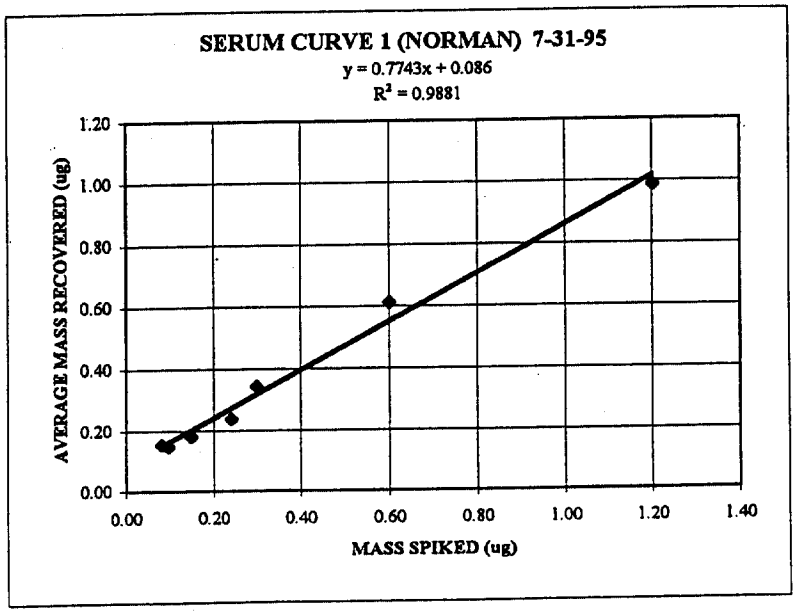
AMDT 111694.1
HWI 6329-134
Norman Cross
Stalar Data

SUMMARY OF 6329-134
SERUM SAMPLES
AMDT 111694.1

Sample ID	Stalar Result (ppm)	DCTSAS final vol (mL)	Qty Sample (mL)	Actual ppm F- in Sample
F52548-2	0.04	2.0	0.10	0.79
F52549-2	0.03	2.0	0.10	0.52
F52559-2	0.03	2.0	0.10	0.52
F52566-2	0.04	2.0	0.10	0.72
F52567-2	0.03	2.0	0.10	0.62
F52548-4	0.02	2.0	0.10	0.46
F52549-4	0.03	2.0	0.10	0.55
F52559-4	0.02	2.0	0.10	0.39
F52566-4	0.02	2.0	0.10	0.34
F52557-4	0.03	2.0	0.10	0.51
F52548-6	0.02	2.0	0.10	0.43
F52549-6	0.02	2.0	0.10	0.40
F52559-6	0.04	2.0	0.10	0.75
F52566-6	0.04	2.0	0.10	0.86
F52567-6	0.03	2.0	0.10	0.65
F52548-8	0.04	2.0	0.10	0.77
F52549-8	0.03	2.0	0.10	0.57
F52559-8	0.02	2.0	0.10	0.35
F52566-8	0.03	2.0	0.10	0.56
F52567-8	0.03	2.0	0.10	0.66
F52548-12	0.02	2.0	0.10	0.44
F52549-12	0.02	2.0	0.10	0.39
F52559-12	ND	2.0	0.10	ND
F52566-12	0.02	2.0	0.10	0.31
F52567-12	0.02	2.0	0.10	0.42
F52548-24	0.11	2.0	0.10	2.26
F52549-24	0.05	2.0	0.10	0.91
F52559-24	0.04	2.0	0.10	0.87
F52566-24	0.04	2.0	0.10	0.77
F52567-24	0.04	2.0	0.10	0.89
F52548-48	0.04	2.0	0.10	0.88
F52549-48	0.04	2.0	0.10	0.83
F52559-48	0.04	2.0	0.10	0.76
F52566-48	0.05	2.0	0.10	0.94
F52567-48	0.04	2.0	0.10	0.72

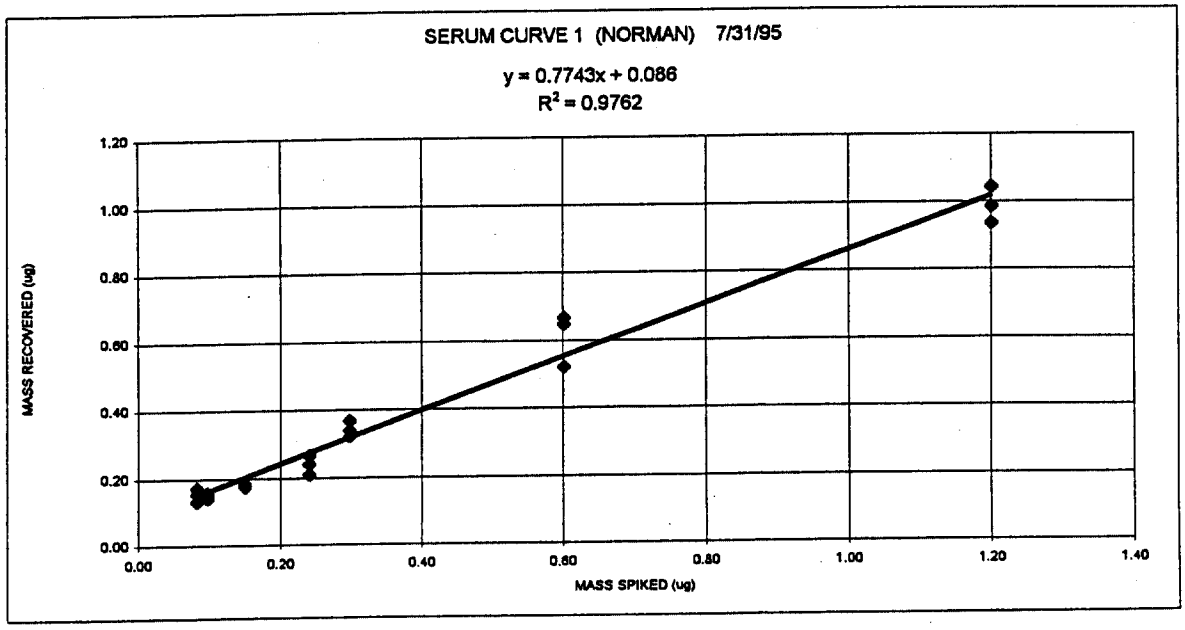
SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Average Mass Recovered (ug F-)	% Recovery
Spk 34-1	0.09	2.0	0.004	34.00			
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.15	188%
Spk 34-3	0.08	2.0	0.004	34.00			
Spk 40-1	0.08	2.0	0.004	40.00			
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.15	155%
Spk 40-3	0.07	2.0	0.004	40.00			
Spk 62-1	0.09	2.0	0.004	62.00			
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	121%
Spk 62-3	0.09	2.0	0.004	62.00			
Spk 100-1	0.11	2.0	0.004	100.0			
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	99%
Spk 100-3	0.13	2.0	0.004	100.0			
Spk 124-1	0.16	2.0	0.004	124.0			
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	115%
Spk 124-3	0.18	2.0	0.004	124.0			
Spk 250-1	0.33	2.0	0.004	250.0			
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.61	102%
Spk 250-3	0.32	2.0	0.004	250.0			
Spk 500-1	0.47	2.0	0.004	500.0			
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%
Spk 500-3	0.52	2.0	0.004	500.0			



SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery		
Spk 34-1	0.09	2.0	0.004	34.00	0.08	0.17	211%	STANDARD DEVIATION :	0.2450
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.13	163%	% RSD :	12.9998
Spk 34-3	0.08	2.0	0.004	34.00	0.08	0.16	191%		
Spk 40-1	0.08	2.0	0.004	40.00	0.10	0.16	164%	STANDARD DEVIATION :	0.0826
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.14	147%	% RSD :	5.3307
Spk 40-3	0.07	2.0	0.004	40.00	0.10	0.15	154%		
Spk 62-1	0.09	2.0	0.004	62.00	0.15	0.18	120%	STANDARD DEVIATION :	0.0263
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	119%	% RSD :	2.1670
Spk 62-3	0.09	2.0	0.004	62.00	0.15	0.18	124%		
Spk 100-1	0.11	2.0	0.004	100.0	0.24	0.21	88%	STANDARD DEVIATION :	0.1138
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	100%	% RSD :	11.4530
Spk 100-3	0.13	2.0	0.004	100.0	0.24	0.27	110%		
Spk 124-1	0.16	2.0	0.004	124.0	0.30	0.32	108%	STANDARD DEVIATION :	0.0778
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	114%	% RSD :	6.7516
Spk 124-3	0.18	2.0	0.004	124.0	0.30	0.37	124%		
Spk 250-1	0.33	2.0	0.004	250.0	0.60	0.67	111%	STANDARD DEVIATION :	0.1318
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.52	87%	% RSD :	12.9196
Spk 250-3	0.32	2.0	0.004	250.0	0.60	0.65	108%		
Spk 500-1	0.47	2.0	0.004	500.0	1.20	0.94	78%	STANDARD DEVIATION :	0.0442
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%	% RSD :	5.3672
Spk 500-3	0.52	2.0	0.004	500.0	1.20	1.04	87%		



1995-08-08 10:58 OutPut of : 950808A1
Operator : DDW
Date of the Analysis : 1995-08-08 07:01
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950808A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DILTISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.44	96%								
2	Drift	1.50	1.47	98%								
3	Wash		ND									
4	Standard 1	0.015	0.015	97%								
5	Standard 2	0.03	0.03	99%								
6	Standard 3	0.06	0.06	103%								
7	Standard 4	0.09	0.09	100%								
8	Standard 5	0.12	0.12	99%								
9	Standard 6	0.15	0.15	100%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.24	103%								
13	Standard 10	1.50	1.46	97%								
14	Drift	1.50	1.53	102%								
15	Wash		ND									
16	SERUM BLK 1		0.12		2.0	0.10	2.46					
17	SERUM BLK 2		0.05		2.0	0.10	1.04					
18	SPK 62-1		0.09		2.0	0.10	1.86	0.004	62.00	0.15	0.19	125%
19	SPK 62-2		0.10		2.0	0.10	1.90	0.004	62.00	0.15	0.19	128%
20	SPK 250-1		0.28		2.0	0.10	5.58	0.004	250.0	0.60	0.56	93%
21	SPK 250-2		0.20		2.0	0.10	3.98	0.004	250.0	0.60	0.40	66%
22	SPK 250-3		0.25		2.0	0.10	5.08	0.004	250.0	0.60	0.51	85%
23	F52548-2		0.04		2.0	0.10	0.79					
24	F52549-2		0.03		2.0	0.10	0.52					
25	F52559-2		0.03		2.0	0.10	0.52					
26	Drift	1.50	1.53	102%								
27	Wash		ND									
28	F52566-2		0.04		2.0	0.10	0.72					
29	F52567-2		0.03		2.0	0.10	0.62					
30	SPK 62-3		0.08		2.0	0.10	1.61	0.004	62.00	0.15	0.16	108%
31	SPK 62-4		0.11		2.0	0.10	2.28	0.004	62.00	0.15	0.23	153%
32	SPK 250-1		0.24		2.0	0.10	4.86	0.004	250.0	0.60	0.49	81%
33	SPK 250-2		0.28		2.0	0.10	5.56	0.004	250.0	0.60	0.56	93%
34	BLK 1		0.10		2.0	0.10	2.03					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol. (mL)	Qty Sample (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc. FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
35	BLK 2		0.06		2.0	0.10	1.11					
36	BLK 3		0.04		2.0	0.10	0.88					
37	BLK 4		0.05		2.0	0.10	0.95					
38	Drift	1.50	1.55	103%								
39	Wash		ND									
40	BLK 5		0.04		2.0	0.10	0.84					
41	SPK 62-1		0.09		2.0	0.10	1.86	0.004	62.00	0.15	0.19	125%
42	SPK 62-2		0.11		2.0	0.10	2.29	0.004	62.00	0.15	0.23	154%
43	SPK 250-1		0.25		2.0	0.10	4.94	0.004	250.0	0.60	0.49	82%
44	SPK 250-2		0.25		2.0	0.10	4.98	0.004	250.0	0.60	0.50	83%
45	SPK 250-3		0.30		2.0	0.10	5.94	0.004	250.0	0.60	0.59	99%
46	SPK 250-4		0.23		2.0	0.10	4.56	0.004	250.0	0.60	0.46	76%
47	BLK		0.12		2.0	0.10	2.34					
48	BLK		0.04		2.0	0.10	0.80					
49	F52548-4		0.02		2.0	0.10	0.46					
50	Drift	1.50	1.56	104%								
51	Wash		ND									
52	F52549-4		0.03		2.0	0.10	0.55					
53	F52559-4		0.02		2.0	0.10	0.39					
54	F52566-4		0.02		2.0	0.10	0.34					
55	F52557-4		0.03		2.0	0.10	0.51					
56	F52548-6		0.02		2.0	0.10	0.43					
57	F52549-6		0.02		2.0	0.10	0.40					
58	F52559-6		0.04		2.0	0.10	0.75					
59	SPK 62-1		0.08		2.0	0.10	1.57	0.004	62.00	0.15	0.16	105%
60	SPK 62-2		0.10		2.0	0.10	2.06	0.004	62.00	0.15	0.21	139%
61	SPK 250-1		0.11		2.0	0.10	2.21	0.004	250.0	0.60	0.22	37%
62	Drift	1.50	1.57	105%								
63	Wash		ND									
64	SPK 250-2		0.20		2.0	0.10	3.96	0.004	250.0	0.60	0.40	66%
65	SPK 250-3		0.31		2.0	0.10	6.16	0.004	250.0	0.60	0.62	103%
66	BLK		0.12		2.0	0.10	2.38					
67	F52566-6		0.04		2.0	0.10	0.86					
68	F52567-6		0.03		2.0	0.10	0.65					
69	Drift	1.50	1.57	105%								
70	Wash		ND									

1995-08-08 14:27 OutPut of : 950808B1
Operator : DDW
Date of the Analysis : 1995-08-08 10:58
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950808B1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DLTISAB final vol (mL)	Qty Sample (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.47	98%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.015	97%								
5	Standard 2	0.03	0.03	99%								
6	Standard 3	0.06	0.06	104%								
7	Standard 4	0.09	0.09	100%								
8	Standard 5	0.12	0.12	98%								
9	Standard 6	0.15	0.15	101%								
10	Standard 7	0.30	0.28	94%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.23	103%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.49	99%								
15	Wash		ND									
16	F52549-8		0.03		2.0	0.10	0.57					
17	F52559-8		0.02		2.0	0.10	0.35					
18	F52566-8		0.03		2.0	0.10	0.56					
19	F52567-8		0.03		2.0	0.10	0.66					
20	F52548-12		0.02		2.0	0.10	0.44					
21	F52549-12		0.02		2.0	0.10	0.39					
22	F52559-12		ND		2.0	0.10	ND					
23	F52566-12		0.02		2.0	0.10	0.31					
24	F52567-12		0.02		2.0	0.10	0.42					
25	SPK 62-1		0.10		2.0	0.10	1.95	0.004	62.00	0.15	0.20	131%
26	Drift	1.50	1.48	99%								
27	Wash		ND									
28	SPK 62-2		0.12		2.0	0.10	2.49	0.004	62.00	0.15	0.25	167%
29	SPK 250-1		0.33		2.0	0.10	6.68	0.004	250.0	0.60	0.67	111%
30	SPK 250-2		0.39		2.0	0.10	7.84	0.004	250.0	0.60	0.78	131%
31	F52548-8		0.04		2.0	0.10	0.77					
32	BLK		0.07		2.0	0.10	1.43					
33	BLK		0.07		2.0	0.10	1.36					
34	BLK		0.04		2.0	0.10	0.84					

AMDT 11169-1
HWI 6329-134 Seq
Norman Counsel

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DIGTISAB final vol. (mL)	Qty Sample (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
35	BLK		0.04		2.0	0.10	0.80					
36	BLK		0.04		2.0	0.10	0.82					
37	SPK 62-1		0.08		2.0	0.10	1.54	0.004	62.00	0.15	0.15	103%
38	Drift	1.50	1.49	99%								
39	Wash		ND									
40	SPK 62-2		0.11		2.0	0.10	2.16	0.004	62.00	0.15	0.22	145%
41	SPK 250-1		0.21		2.0	0.10	4.28	0.004	250.0	0.60	0.43	71%
42	SPK 250-2		0.21		2.0	0.10	4.12	0.004	250.0	0.60	0.41	69%
43	SPK 250-3		0.23		2.0	0.10	4.68	0.004	250.0	0.60	0.47	78%
44	SPK 250-4		0.28		2.0	0.10	5.66	0.004	250.0	0.60	0.57	94%
45	SPK 250-5		0.25		2.0	0.10	5.06	0.004	250.0	0.60	0.51	84%
46	BLK		0.15		2.0	0.10	2.90					
47	BLK		ND		2.0	0.10	ND					
48	F52548-24		0.11		2.0	0.10	2.26					
49	F52549-24		0.05		2.0	0.10	0.91					
50	Drift	1.50	1.49	99%								
51	Wash		ND									
52	F52559-24		0.04		2.0	0.10	0.87					
53	F52566-24		0.04		2.0	0.10	0.77					
54	F52567-24		0.04		2.0	0.10	0.89					
55	F52548-48		0.04		2.0	0.10	0.88					
56	F52549-48		0.04		2.0	0.10	0.83					
57	F52559-48		0.04		2.0	0.10	0.76					
58	F52566-48		0.05		2.0	0.10	0.94					
59	F52567-48		0.04		2.0	0.10	0.72					
60	BLK		0.08		2.0	0.10	1.58					
61	BLK		0.06		2.0	0.10	1.13					
62	Drift	1.50	1.49	99%								
63	Wash		ND									
64	BLK		0.06		2.0	0.10	1.21					
65	BLK		0.06		2.0	0.10	1.23					
66	BLK		0.05		2.0	0.10	1.06					
67	SPK 62-1		0.10		2.0	0.10	1.93	0.004	62.00	0.15	0.19	129%
68	Drift	1.50	1.48	99%								
69	Wash		ND									

DRW 8/25/95
AMBT 111694.1
HWI 6329-134 Sam
Norman Cmnt 1

1995-08-08 10:58 OutPut of : 950808A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-08 07:01

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950808A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00074
a0 = -1.18614

Fluoride L
Calibration order = 2

Correlation : r = 0.99946

Result = a2 * x^2 + a1 * x + a0

a2 = -0.00000
a1 = 0.00030
a0 = 0.00019

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file :	. TXT
	Reproces	: No

10

1995-08-08 10:58 OutPut of : 950808A1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

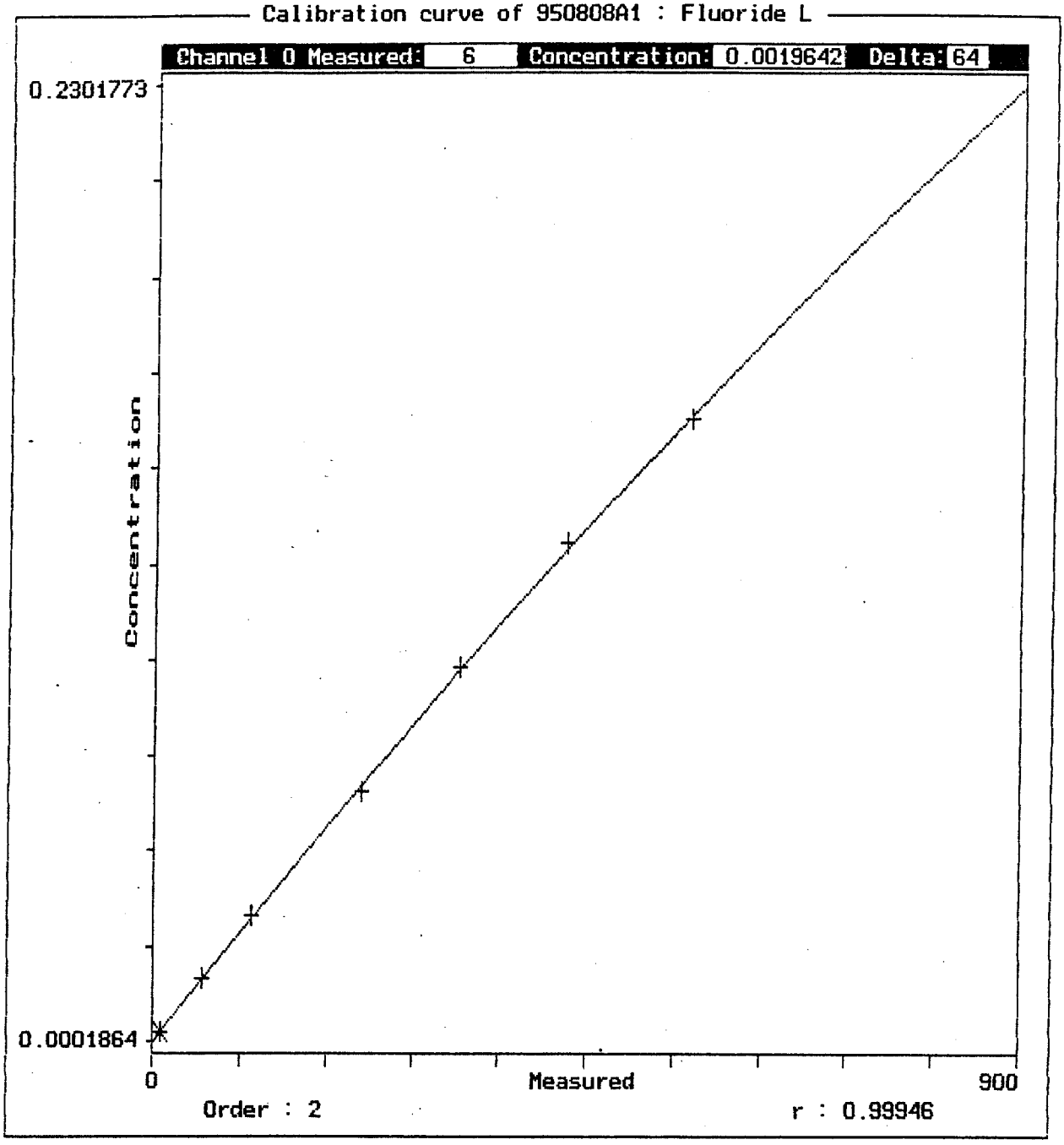
Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

1995-08-08 10:58 OutPut of : 950808A1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result F	Time	Ch	Result F	Time
wt	iw	Initial Wash	3	0.065	65	4	0.0002	0
1	t	Tracer	3	1.436	209	4	0.4779	0
2	d	Drift	3	1.468	383	4	0.4793	0
3	w	Wash	3	0.065	620	4	0.0002	0
4	s1	Standard 1	3	0.071	734	4	0.0146	0
5	s2	Standard 2	3	0.077	909	4	0.0297	0
6	s3	Standard 3	3	0.093	1081	4	0.0618	0
7	s4	Standard 4	3	0.109	1259	4	0.0896	0
8	s5	Standard 5	3	0.129	1433	4	0.1185	0
9	s6	Standard 6	3	0.156	1609	4	0.1507	0
10	s7	Standard 7	3	0.279	1783	4	0.2466	0
11	s8	Standard 8	3	0.617	1959	4	0.3715	0
12	s9	Standard 9	3	1.239	2133	4	0.4644	0
13	s10	Standard 10	3	1.460	2307	4	0.4790	0
14	d	Drift	3	1.532	2483	4	0.4816	0
15	w	Wash	3	0.065	2725	4	0.0002	0
16	u	SERUM BLK 1	3	0.133	2834	4	0.1231	0
17	u	SERUM BLK 2	3	0.088	3008	4	0.0521	0
18	u	SPK 62-1	3	0.112	3182	4	0.0931	0
19	u	SPK 62-2	3	0.113	3360	4	0.0950	0
20	u	SPK 250-1	3	0.279	3536	4	0.2470	0
21	u	SPK 250-2	3	0.199	3710	4	0.1908	0
22	u	SPK 250-3	3	0.254	3886	4	0.2317	0
23	u	F52548-2	3	~~0.082~~	4060	4	0.0397	0
24	u	F52549-2	3	0.076	4234	4	0.0259	0
25	u	F52559-2	3	0.076	4408	4	0.0259	0
26	d	Drift	3	1.533	4584	4	0.4816	0
27	w	Wash	3	0.065	4825	4	0.0002	0
28	u	F52566-2	3	0.080	4926	4	0.0360	0
29	u	F52567-2	3	0.078	5109	4	0.0311	0
30	u	SPK 62-3	3	0.104	5284	4	0.0805	0
31	u	SPK 62-4	3	0.126	5463	4	0.1141	0
32	u	SPK 250-1	3	0.243	5636	4	0.2239	0
33	u	SPK 250-2	3	0.278	5811	4	0.2460	0
34	u	BLK 1	3	0.117	5985	4	0.1013	0
35	u	BLK 2	3	0.090	6161	4	0.0554	0
36	u	BLK 3	3	0.084	6337	4	0.0442	0
37	u	BLK 4	3	0.085	6511	4	0.0473	0
38	d	Drift	3	1.548	6687	4	0.4819	0
39	w	Wash	3	0.065	6917	4	0.0002	0
40	u	BLK 5	3	0.083	7035	4	0.0419	0
41	u	SPK 62-1	3	0.112	7212	4	0.0931	0
42	u	SPK 62-2	3	0.126	7388	4	0.1146	0
43	u	SPK 250-1	3	0.247	7562	4	0.2272	0
44	u	SPK 250-2	3	0.249	7738	4	0.2282	0
45	u	SPK 250-3	3	0.297	7912	4	0.2569	0
46	u	SPK 250-4	3	0.228	8087	4	0.2136	0
47	u	BLK	3	0.128	8263	4	0.1170	0
48	u	BLK	3	0.082	8437	4	0.0402	0
49	u	F52548-4	3	0.074	8613	4	0.0228	0
50	d	Drift	3	1.557	8787	4	0.4821	0
51	w	Wash	3	0.065	9019	4	0.0002	0
52	u	F52549-4	3	0.076	9136	4	0.0277	0
53	u	F52559-4	3	0.073	9305	4	0.0193	0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	F52566-4	3	0.072	9488	4	0.0169	0
55	u	F52557-4	3	0.075	9664	4	0.0254	0
56	u	F52548-6	3	0.074	9834	4	0.0216	0
57	u	F52549-6	3	0.073	10013	4	0.0201	0
58	u	F52559-6	3	0.081	10190	4	0.0377	0
59	u	SPK 62-1	3	0.102	10364	4	0.0783	0
60	u	SPK 62-2	3	0.118	10540	4	0.1032	0
61	u	SPK 250-1	3	0.124	10715	4	0.1107	0
62	d	Drift	3	1.571	10889	4	0.4823	0
63	w	Wash	3	0.065	11100	4	0.0002	0
64	u	SPK 250-2	3	0.198	11239	4	0.1899	0
65	u	SPK 250-3	3	0.308	11415	4	0.2627	0
66	u	BLK	3	0.130	11591	4	0.1190	0
67	u	F52566-6	3	0.083	11764	4	0.0431	0
68	u	F52567-6	3	0.078	11940	4	0.0323	0
69	d	Drift	3	1.572	12115	4	0.4823	0
70	w	Wash	3	0.065	12351	4	0.0002	0
wt	rw	RunOut Wash	3	0.065	12590	4	0.0002	0


Calibration curve of 950808A1 : Fluoride L
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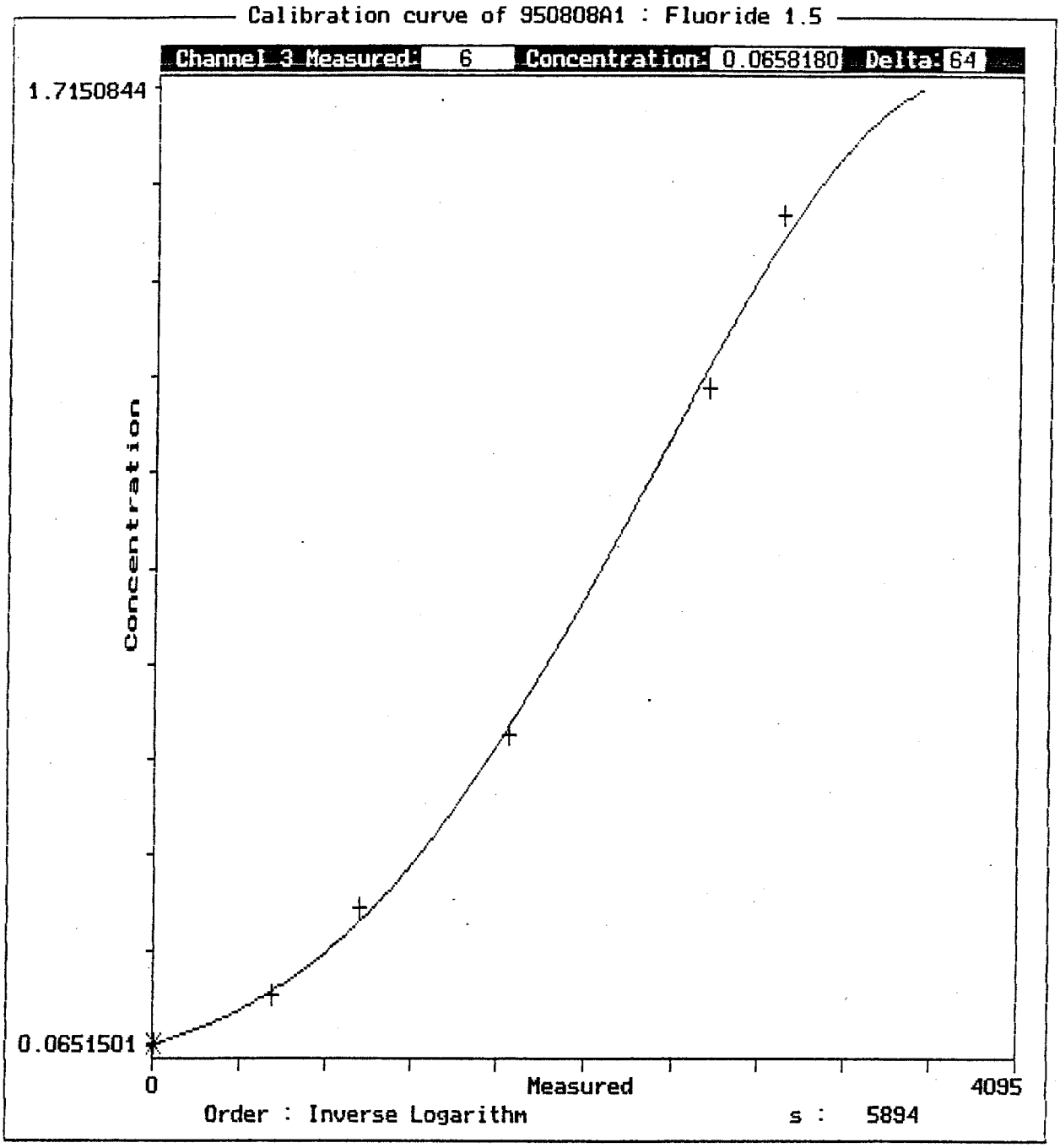
Delta: 64
0.2301773
0.0001864
Concentration
0
Measured
900
Order : 2
r : 0.99946


Calibration curve of 950808A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0658180
Delta: 64
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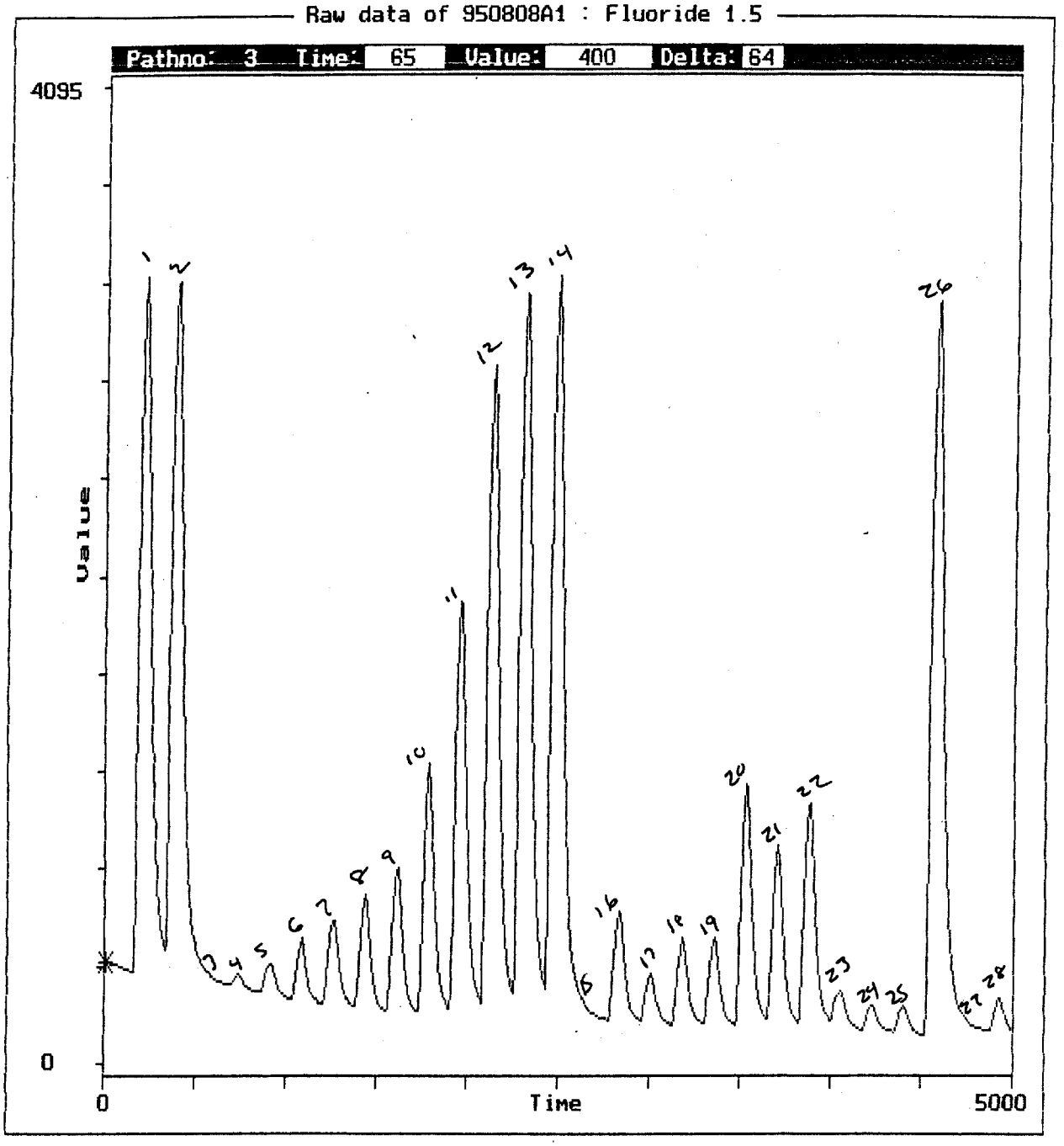
0.0651501
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5894



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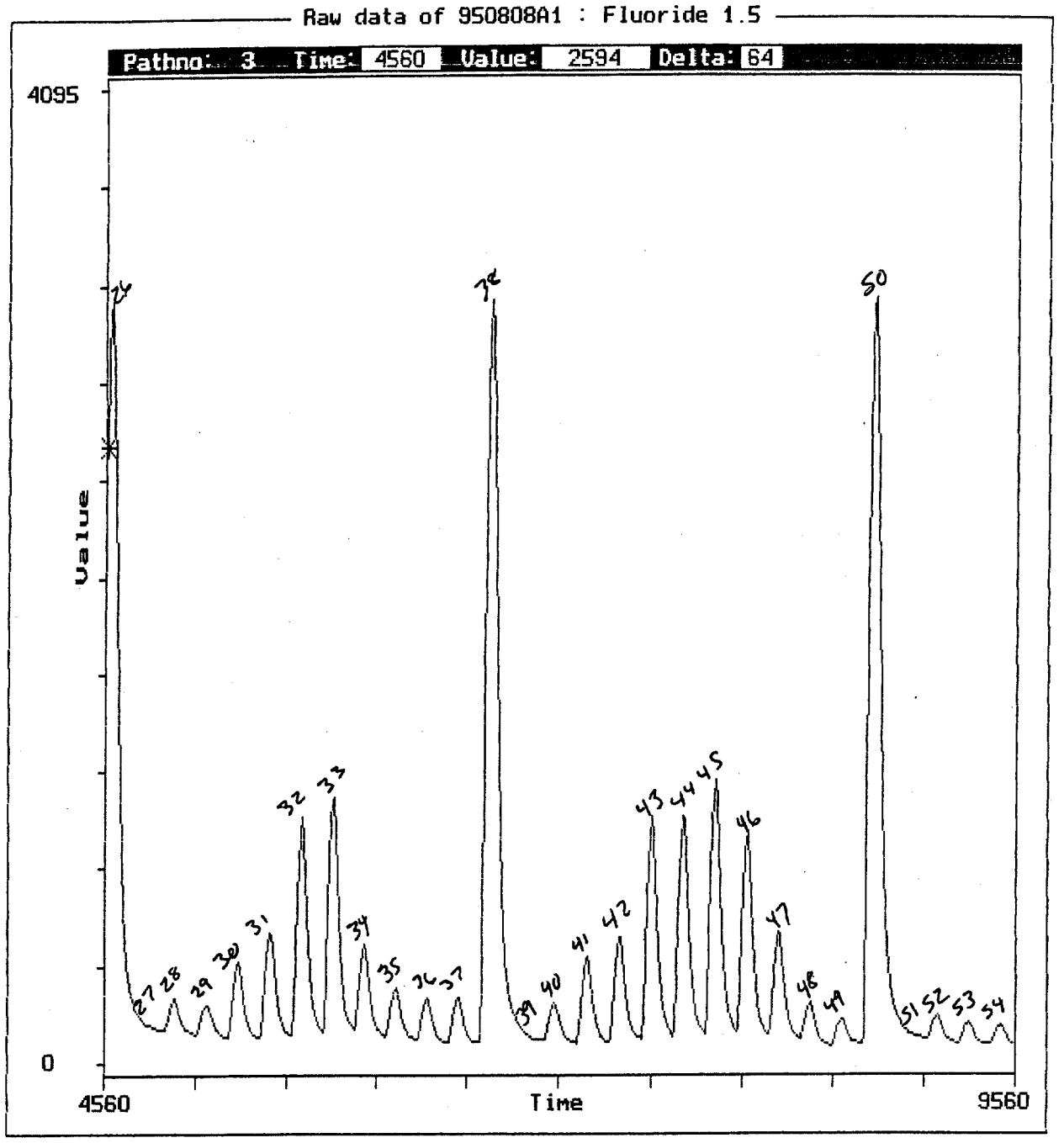
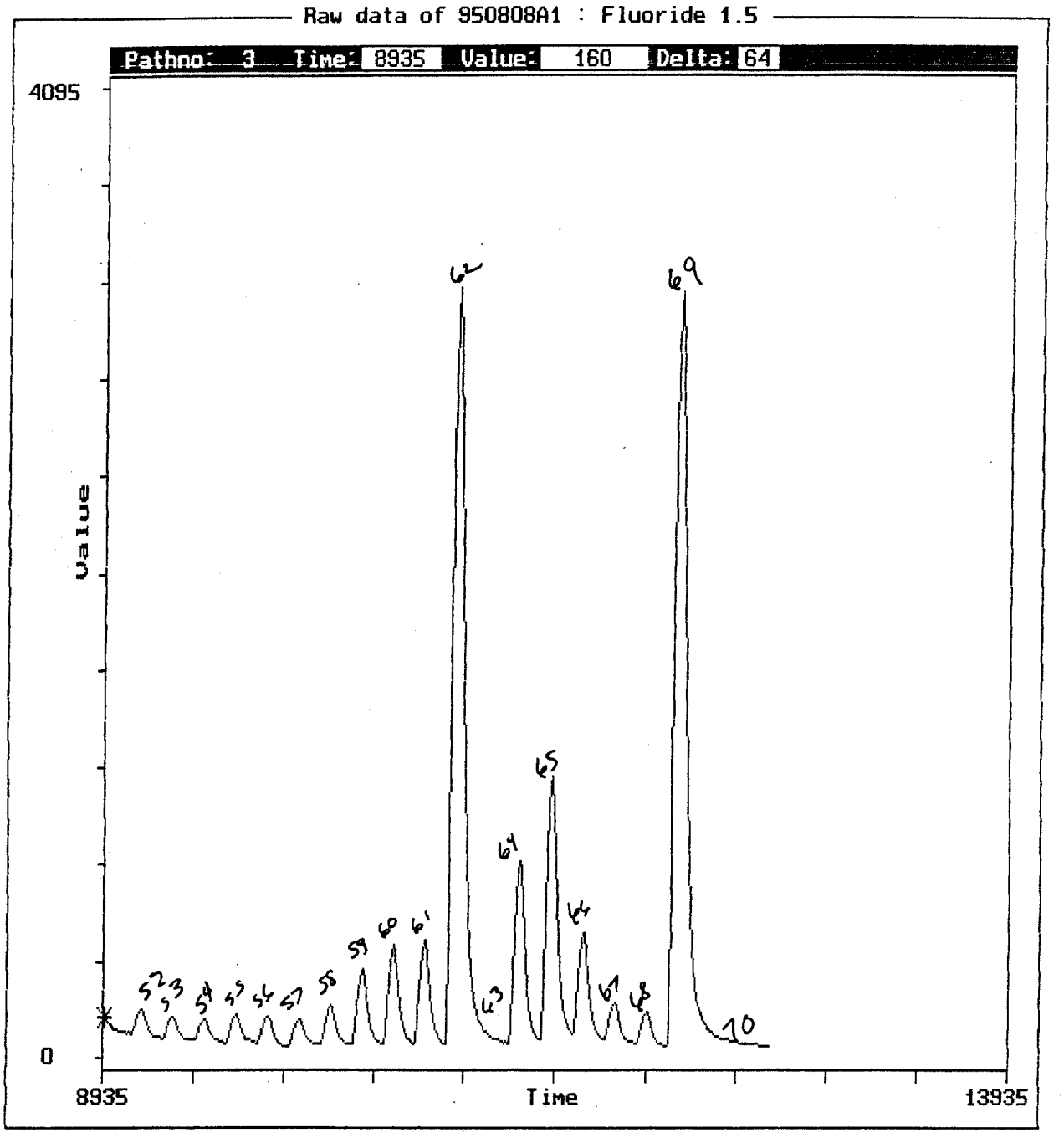

Raw data of 950808A1 : Fluoride 1.5
Pathno: 3 Time: 4560 Value: 2594 Delta: 64
4095
0
Value
4560
Time
9560
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

DDW 8/25/95
AMDT 111694.1
HWI 6329-134 Sa
Norman Cmt 1

1995-08-08 14:27 OutPut of : 950808B1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-08 10:58

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950808B1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00069
a0 = -1.21965

Fluoride L
Calibration order = 2

Correlation : r = 0.99948

Result = a2 * x^2 + a1 * x + a0

a2 = 0.00000
a1 = 0.00023
a0 = 0.00544

Sampler
Type : SA1000
Number : 1
Sample Time : 50 sec.
Wash Time : 120 sec.
Air Time : 1 sec.
Take up : Single
sPecial : None
needle Height : 70 mm.

Diluter
needle Height : 80 mm
dilution Factor : 10
dilution Volume : 2.5 ml.
Resample : 1
Dilution runs : 1

User file : . TXT
Reproces : No

1995-08-08 14:27 OutPut of : 950808B1

Fluoride 1.5

Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

Fluoride L

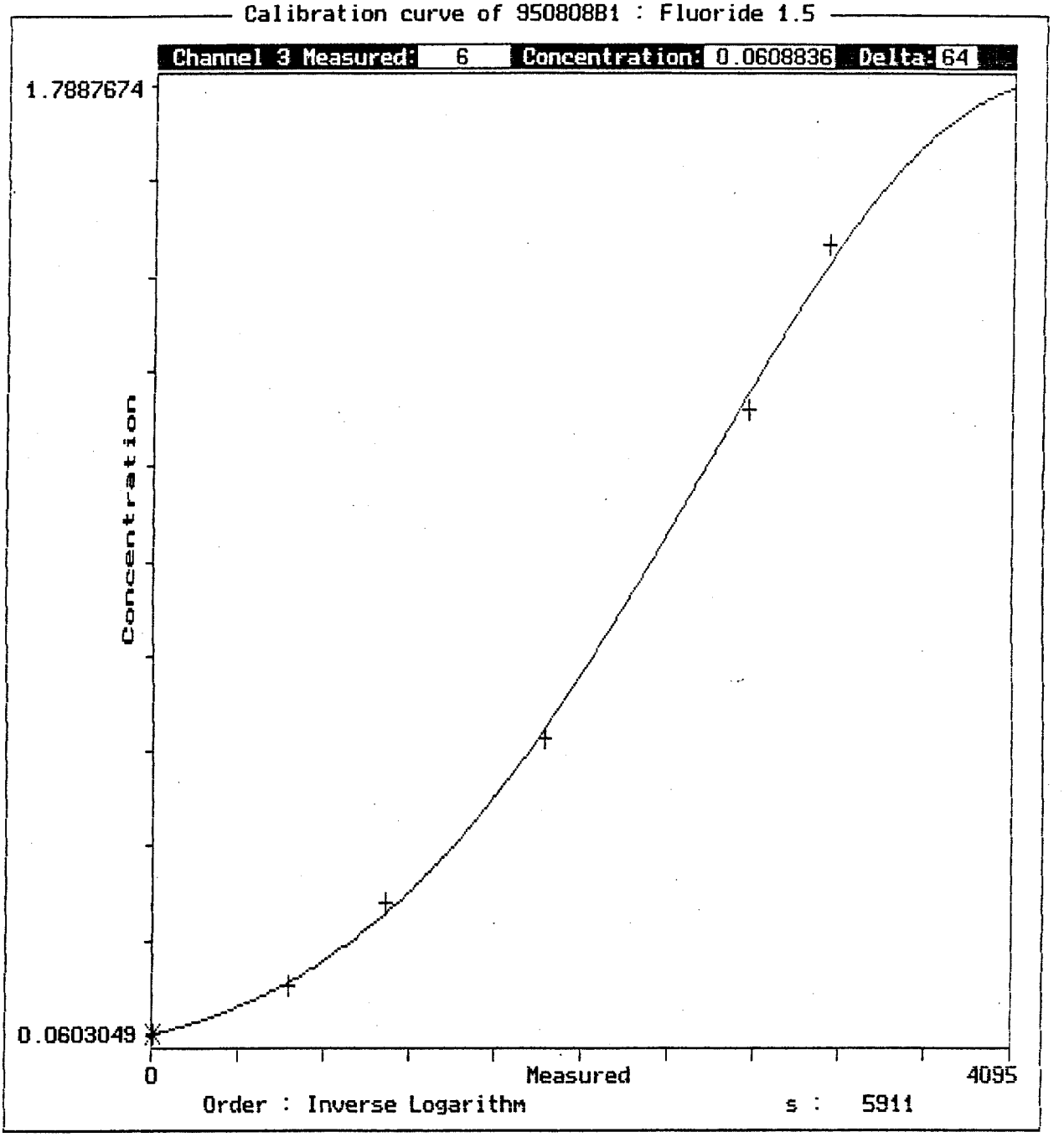
Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

1995-08-08 14:27 OutPut of : 950808B1

```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger Limit   : 1800 Sec
Peak shape      : Pointed
stArt ignore    : 60   Sec
eNd   ignore    : 120  Sec
Measure window  : 75   %
Filter          : No
Regeneration    : No
formUla    : c4:=c3
output     : #.####
```

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iw	Initial Wash	3	0.060	65	4	0.0054	0
1	t	Tracer	3	1.468	210	4	0.7420	0
2	d	Drift	3	1.480	386	4	0.7466	0
3	w	Wash	3	0.060	628	4	0.0054	0
4	s1	Standard 1	3	0.064	737	4	0.0145	0
5	s2	Standard 2	3	0.071	911	4	0.0297	0
6	s3	Standard 3	3	0.089	1085	4	0.0621	0
7	s4	Standard 4	3	0.107	1261	4	0.0904	0
8	s5	Standard 5	3	0.126	1437	4	0.1172	0
9	s6	Standard 6	3	0.155	1613	4	0.1512	0
10	s7	Standard 7	3	0.281	1787	4	0.2577	0
11	s8	Standard 8	3	0.616	1961	4	0.4307	0
12	s9	Standard 9	3	1.230	2137	4	0.6533	0
13	s10	Standard 10	3	1.468	2311	4	0.7417	0
14	d	Drift	3	1.487	2487	4	0.7496	0
15	w	Wash	3	0.060	2729	4	0.0054	0
16	u	F52549-8	3	0.071	2840	4	0.0283	0
17	u	F52559-8	3	0.066	3010	4	0.0174	0
18	u	F52566-8	3	0.071	3188	4	0.0281	0
19	u	F52567-8	3	0.073	3362	4	0.0328	0
20	u	F52548-12	3	0.068	3534	4	0.0220	0
21	u	F52549-12	3	0.067	3712	4	0.0195	0
22	u	F52559-12	3	0.064	3878	4	0.0134	0
23	u	F52566-12	3	0.065	4063	4	0.0156	0
24	u	F52567-12	3	0.067	4235	4	0.0208	0
25	u	SPK 62-1	3	0.112	4413	4	0.0977	0
26	d	Drift	3	1.480	4589	4	0.7468	0
27	w	Wash	3	0.060	4824	4	0.0054	0
28	u	SPK 62-2	3	0.132	4939	4	0.1247	0
29	u	SPK 250-1	3	0.334	5113	4	0.2919	0
30	u	SPK 250-2	3	0.392	5289	4	0.3253	0
31	u	F52548-8	3	0.076	5459	4	0.0387	0
32	u	BLK	3	0.095	5642	4	0.0716	0
33	u	BLK	3	0.092	5814	4	0.0682	0
34	u	BLK	3	0.078	5990	4	0.0419	0
35	u	BLK	3	0.076	6164	4	0.0399	0
36	u	BLK	3	0.077	6340	4	0.0412	0
37	u	SPK 62-1	3	0.098	6514	4	0.0770	0
38	d	Drift	3	1.489	6690	4	0.7503	0
39	w	Wash	3	0.060	6929	4	0.0054	0
40	u	SPK 62-2	3	0.119	7040	4	0.1081	0
41	u	SPK 250-1	3	0.214	7216	4	0.2072	0
42	u	SPK 250-2	3	0.206	7390	4	0.1997	0
43	u	SPK 250-3	3	0.234	7565	4	0.2231	0
44	u	SPK 250-4	3	0.283	7741	4	0.2589	0
45	u	SPK 250-5	3	0.253	7915	4	0.2377	0
46	u	BLK	3	0.150	8089	4	0.1451	0
47	u	BLK	3	0.061	8241	4	0.0066	0
48	u	F52548-24	3	0.123	8439	4	0.1129	0
49	u	F52549-24	3	0.080	8610	4	0.0457	0
50	d	Drift	3	1.488	8788	4	0.7498	0
51	w	Wash	3	0.060	9015	4	0.0054	0
52	u	F52559-24	3	0.079	9138	4	0.0437	0
53	u	F52566-24	3	0.076	9310	4	0.0387	0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	F52567-24	3	0.079	9487	4	0.0446	0
55	u	F52548-48	3	0.079	9662	4	0.0439	0
56	u	F52549-48	3	0.077	9837	4	0.0417	0
57	u	F52559-48	3	0.075	10013	4	0.0378	0
58	u	F52566-48	3	0.080	10187	4	0.0471	0
59	u	F52567-48	3	0.074	10361	4	0.0358	0
60	u	BLK	3	0.099	10539	4	0.0788	0
61	u	BLK	3	0.085	10706	4	0.0564	0
62	d	Drift	3	1.487	10886	4	0.7496	0
63	w	Wash	3	0.060	11127	4	0.0054	0
64	u	BLK	3	0.088	11236	4	0.0607	0
65	u	BLK	3	0.088	11412	4	0.0614	0
66	u	BLK	3	0.084	11586	4	0.0532	0
67	u	SPK 62-1	3	0.111	11760	4	0.0963	0
68	d	Drift	3	1.482	11935	4	0.7475	0
69	w	Wash	3	0.060	12166	4	0.0054	0
wt	rw	RunOut Wash	3	0.060	12410	4	0.0054	0


Calibration curve of 950808B1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0608836
Delta: 64
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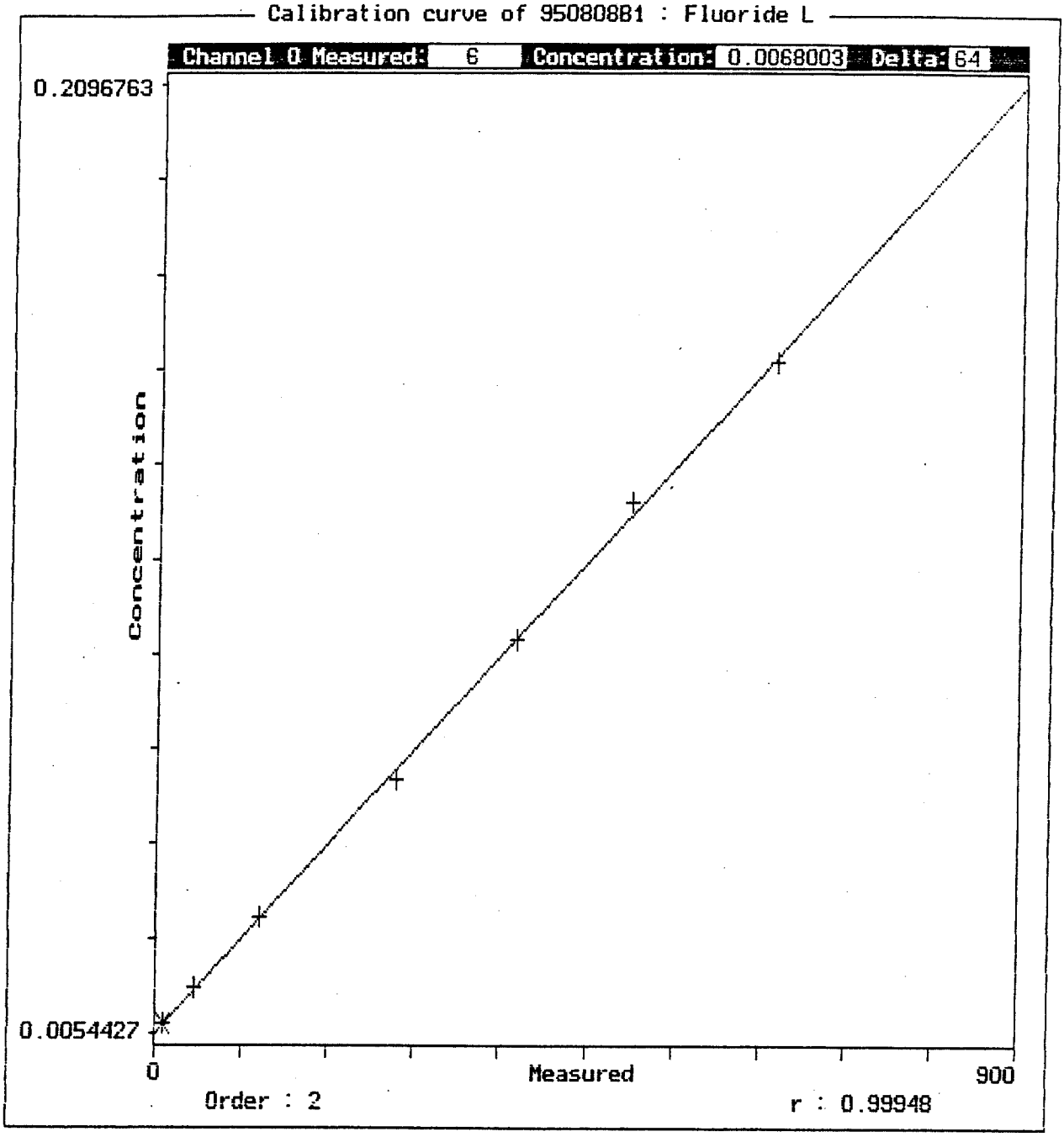
Concentration
0.0603049
0
Measured
4095
Order : Inverse Logarithm
s : 5911

000932a


Calibration curve of 95080881 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0068003
Delta: 64
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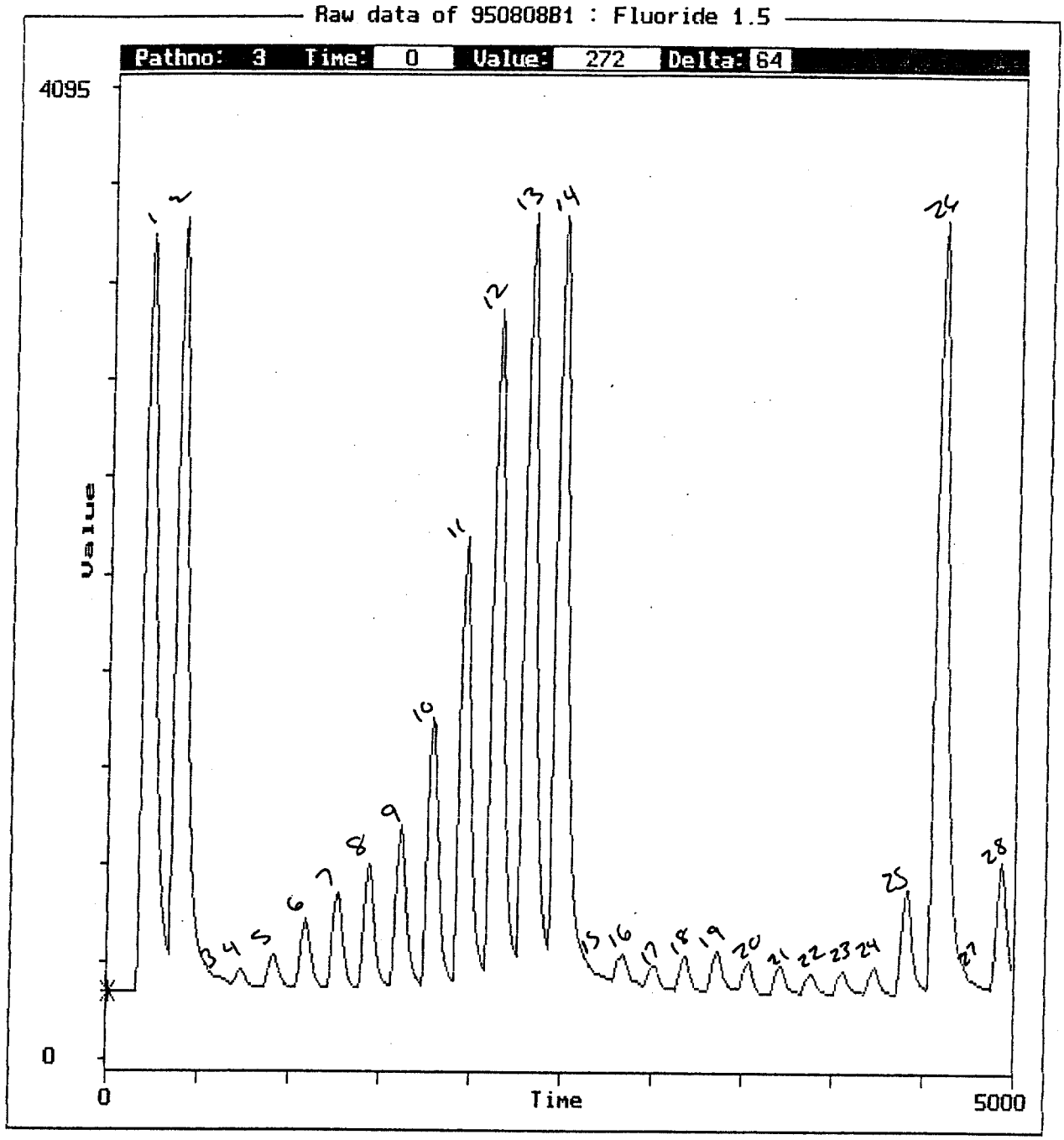
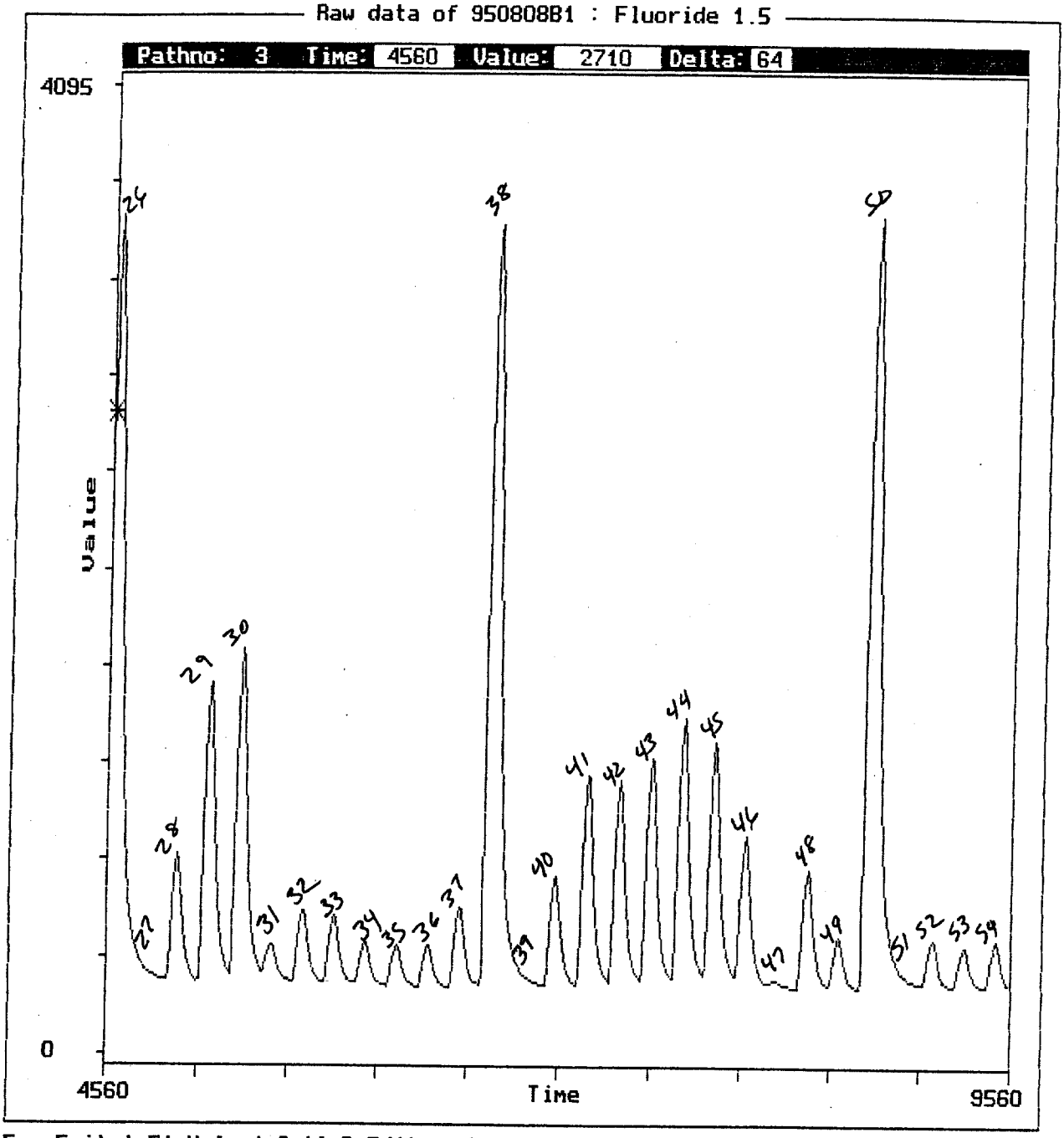
0.0054427
Concentration
0
Measured
900
Order : 2
r : 0.99948



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦


Raw data of 950808B1 : Fluoride 1.5
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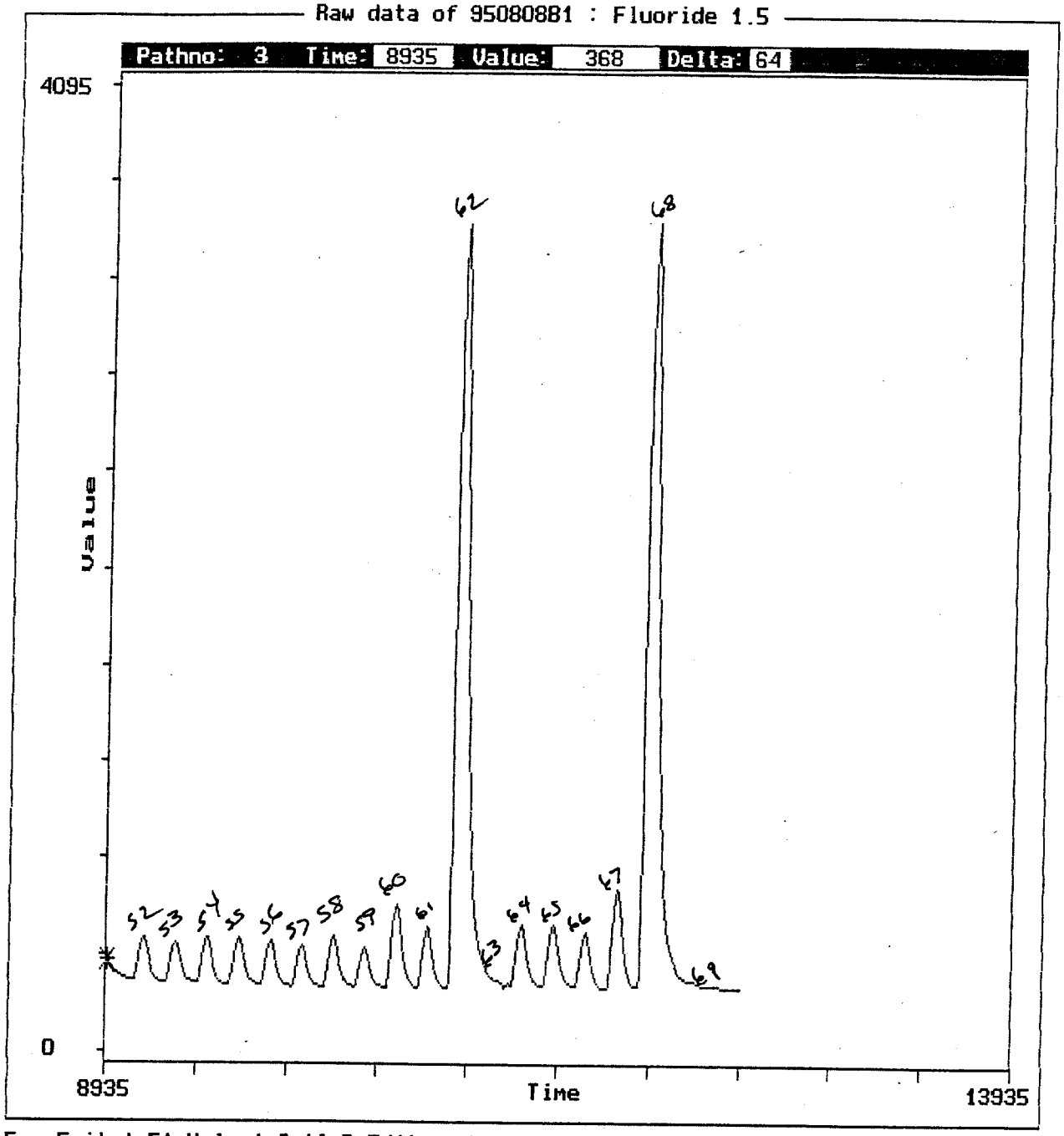
4095
0
Value
4560
Time
9560
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

6. N-EtFOSA	N-ethyl perfluorooctanesulfonamide

Acute Toxicity

1) Final Report, Acute Ocular Irritation Test with T-3608 in Albino Rats, Riker Laboratories, Inc., 3M Reference FX-12, Study No. 0984EB0367, September 5, 1984

2) Final Report, Primary Skin Irritation Test with T-3608 in Albino Rats, Riker Laboratories, Inc., 3M Reference FX-12, Study No. 0984EB0368, August 13, 1984

3) Final Report, Acute Oral Toxicity Screen with T-3066CoC in Albino Rats, Riker Laboratories, Inc., 3M Ref. No. FX-12, Study No. 0981AR0146, July 13, 1981

Acute Toxicity Studies Not Submitted (Bibliography Only)

1) Final Report, Acute Oral Toxicity Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101149, January 31, 1997

2) Final Report, Primary Dermal Irritation/Corrosion Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101150, January 31, 1997

3) Final Report, Primary Eye Irritation/Corrosion Study of T-6684 in Rats (OECD Guidelines), Corning Hazelton Inc., 3M Ref. No. L-14394 (slurry), Study No. CHW 61101151, January 31, 1997

Genotoxicity

1) Final Report, Protocol and two amendments, Mutagenicity Test on T-6294 in an In Vivo Mouse Micronucleus Assay, Corning Hazelton, Inc., Study No. 1785-0-455, May 10, 1996

Previously submitted with May 4, 2000 letter, Advanced Bioanalytical Services, Inc., Analytical Report, Additional Characterization of Metabolites of T-6292, T-6293 and T-6294 from Rat and Human Hepatocytes by TurboIonSpray LC/MS and LC/MS/MS. Semi-Quantitative Analysis of T-6295 in Rat and Human Hepatocytes Incubated with T-6292, T-6293 and T-6294 by LC/MS/MS, January 28, 1998, Report 98AGKP01.3M

Attachments to Letter to C. Auer dated May 18, 2000
Studies and Other Information on Certain
Perfluorooctane Sulfonate-Related Compounds

Mechanistic

1) T. J. Cross and R. G. Schnellmann, Mechanism of Toxicity of a Unique Pesticide N-Ethylperfluorooctane Sulfanomide (NEPFOS), and its metabolite perfluorooctane Sulfonamide (PFOS) to Isolated Rabbit Renal Cortical Mitochondria (RCM), Abstract from 1989 Society of Toxicology Meeting

Previously submitted with May 4, 2000 letter - Qualitative Investigation of the In Vitro Metabolism of T-6292 (n-ethyl FOSE), T-6293 (n-ethyl FOSE phosphate diammonium salt(ester)), T-6294 (n-ethyl perfluorooctane sulfonamide) and T-6295 (perflurooctane sulfonate) by Rat and Human Hepatocytes Using Ion Spray LC/MS and LC/MS/MS, Advanced Bioanalytical Services, Inc., [Preliminary] Analytical Report, Report 96ADEM01.3M, November 12, 1996

Previously submitted with May 4, 2000 letter - Advanced Bioanalytical Services, Inc., Analytical Report, Additional Characterization of Metabolites of T-6292, T-6293 and T-6294 from Rat and Human Hepatocytes by TurboIonSpray LC/MS and LC/MS/MS. Semi-Quantitative Analysis of T-6295 in Rat and Human Hepatocytes Incubated with T-6292, T-6293 and T-6294 by LC/MS/MS, January 28, 1998, Report 98AGKP01.3M

Analytical

1) Analytical and Research Properties – 3M Industrial Hygiene Laboratory, January 1993

6. N-EtFOSA	N-ethyl perfluorooctanesulfonamide

Bibliography Showing Studies in 3M's Possession Believed To Be In FIFRA Docket.

REDACTED

Acute Ocular Irritation Test

with T-3608

in Albino Rabbits

Experiment No.: 0984EB0367

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: July 23, 1984 to July 30, 1984

Conducted By:

G. E. Hart 9/5/84
Date
Sr. Laboratory Technician
Acute Toxicology

K. D. O'Malley, BS 9/5/84
Date
Senior Toxicologist
Study Director

dc: K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

The results of the acute ocular irritation test conducted from July 23, 1984 to July 30, 1984 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3608 is minimally irritating (10.3/110.0) to the eye of the female albino rabbit. Slight conjunctivitis was noted at the one hour evaluation and subsided by the three day evaluation. Neither corneal opacity nor iritis were noted during the seven day test period.

Introduction

The objective of this study was to determine the acute ocular irritation properties of T-3608 when instilled into the eye of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administrations's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits of the New Zealand breed[a] were used to evaluate the ocular irritating properties of the test article. The test method was modeled after that of Draize et al[b].

The test article was instilled into the conjunctival sac of the right eye of each rabbit according to the treatment procedure presented in Table 1 with the left eye of each animal serving as a control. At each scoring interval, the cornea, iris and palpebral conjunctiva were examined and graded for irritation and injury according to a standard scoring system[b]. The maximum possible score at any one examination and scoring period 110 points, which indicates maximal irritation and damage to all three ocular tissues (cornea, iris, conjunctiva) while a score of zero indicates no irritation (Table 2). In this scoring system, special emphasis is placed upon irritation or damage to the cornea, while less emphasis is placed upon damage to the iris and conjunctiva.

After completion of the test, the scores were analyzed, and a descriptive eye irritation rating was assigned to the test article. The criteria used for assignment of the descriptive rating were the frequency, the extent and the persistence of irritation or damage which occurred to the three ocular tissues (Table 3). The individual results are presented in Table 4.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).

The rating is arrived at by selecting the maximum mean irritation score at one hour, one, two or three days after instillation. If the rate of dissipation of injury does not meet the requirements defined for the descriptive rating appropriate for a particular numerical score, the descriptive rating is raised by one or more levels. The rating system is presented in Table 3. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Eye Irritation Test - Albino Rabbits

Treatment Procedure

Test Article	Number of Animals Evaluated	Form Administered	Quantity of Test Article Administered	Contact Period (seconds)	Volume of Wash (tap water)	Evaluation Time Post Dose Administration
T-3608	6	powder	0.1 gm	unlimited	none	1 Hour, 1, 2, 3 and 7 Days

Table 2

Eye Irritation Test - Albino Rabbits

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Draize Grade
Conjunctiva	Redness (A)	
	Redness (refers to palpebral conjunctiva only). Vessels definitely injected above normal.	1
	More diffuse, deeper crimson red, individual vessels not easily discernible.	2
	Diffuse beefy red.	3
	Chemosis (B)	
	Any swelling above normal (included nictitating membrane).	1
	Obvious swelling with partial eversion of the lids.	2
	Swelling with lids about half-closed.	3
	Swelling with lids about half-closed to completely closed.	4
	Discharge (C)	
	Any amount different from normal (Does not include small amount observed in inner canthus of normal animals).	1
	Discharge with moistening of the lids and hairs just adjacent to the lids.	2
	Discharge with moistening of the lids and hairs and considerable area around eye.	3
	Score (A + B + C) x 2 Total maximum = 20	
Cornea	Opacity (A)	
	Opacity - Degree of density (area which is most dense is taken for reading).	
	Scattered or diffuse area, details of iris clearly visible.	1
	Easily discernible translucent areas, details of iris slightly obscured.	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible.	3
	Opaque, iris invisible.	4
	Area of Cornea Involved (B)	
	One quarter (or less) but not zero.	1
	Greater than one-quarter, but less than one-half.	2
	Greater than one-half, but less than three-quarters.	3
	Greater than three-quarters, up to whole area.	4
	Score equals A x B x 5 Total maximum = 80	
Iris	Values (A)	
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive).	1
	No reaction to light, hemorrhage, gross destruction (any or all of these).	2
	Score equals A x 5 Total maximum = 10	

Note: The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva.

Table 3

Eye Irritation Test - Albino Rabbits

Classification of Test Materials
Based on Eye Irritation Properties

Rating	Range	Definition
Non-Irritating	0.0 - 0.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Practically Non-Irritating	>0.5 - 2.5	To maintain this rating, all scores by the one day reading must be zero; otherwise, increase rating one level.
Minimally Irritating	>2.5 - 15.0	To maintain this rating, all scores by the three day reading must be zero; otherwise, increase rating one level.
Mildly Irritating	>15.0 - 25.0	To maintain this rating, all scores by the seven day reading must be zero; otherwise, increase rating one level.
Moderately Irritating	>25.0 - 50.0	To maintain this rating, scores by seven days must be $\leq$ 10 for 60% or more of the animals. Also, mean seven day score must be $\leq$ 20. If seven day mean score is $\leq$ 20 but < 60% of animals show scores < 10, then no animal among those showing scores > 10 can exceed a score of 30 if rating is to be maintained; otherwise, raise rating one level.
Severely Irritating	>50.0 - 80.0	To maintain this rating, scores by seven days must be $\leq$ 30 for 60% or more of the animals. Also, mean seven day score must be $\leq$ 40. If seven day mean score is $\leq$ 40 but < 60% of the animals show scores $\leq$ 30, then no animal among those showing scores > 30 can exceed a score of 60 if rating is to be maintained; otherwise, raise rating one level.
Extremely Irritating	>80.0 - 110.0	

Table 4

Eye Irritation Test - Albino Rabbits

with T-3608

RESULTS

Tissue	Examination Period	ANIMAL NUMBERS 4B1141	4B1144	4B1136	4B1139	4B1142	4B1145	Means
Cornea(D-A)	1 Hour	0	0	0	0	0	0	0.0
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		8(2-2-0)	10(2-2-1)	12(3-2-1)	14(3-2-2)	8(2-1-1)	10(2-2-1)	10.3
	Total	8	10	12	14	8	10	10.3
Cornea(D-A)	1 Day	0	0	0	0	0	0	0.0
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		6(2-1-0)	4(2-0-0)	8(2-2-0)	8(2-1-1)	4(2-0-0)	6(2-1-0)	6.0
	Total	6	4	8	8	4	6	6.0
Cornea(D-A)	2 Days	0	0	0	0	0	0	0.0
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		4(1-1-0)	2(1-0-0)	2(1-0-0)	4(1-1-0)	0	2(1-0-0)	2.3
	Total	4	2	2	4	0	2	2.3
Cornea(D-A)	3 Days	0	0	0	0	0	0	0.0
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		0	0	0	0	0	0	0.0
	Total	0	0	0	0	0	0	0.0
Cornea(D-A)	7 Days	0	0	0	0	0	0	0.0
Iris		0	0	0	0	0	0	0.0
Conjunctiva (RSD)		0	0	0	0	0	0	0.0
	Total	0	0	0	0	0	0	0.0

Key: Cornea: D=Density A=Area

Conjunctiva: R=Redness S=Swelling

Riker Experiment No.: 0984EB0367

APPENDIX I
PROTOCOL

8.

TEST: Acute Ocular Irritation Test

SPONSOR: 3M Commercial Chemicals Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-3608

CONTROL ARTICLE: N/A

PROPOSED STARTING/COMPLETION DATE OF TEST: 7/84 - 10/84

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hamilton Dutchland
Denver Pa

OBJECTIVE: The objective of this test will be to determine the irritation potential of the test article to the ocular tissues (cornea, iris and conjunctiva) of 6 albino rabbits. Rabbits were selected as the test system for their sensitivity to irritants, historical use, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag which will correspond to a card affixed to the outside of the cage. The test article will be instilled into the conjunctival sac of the right eye at a dose of 0.1 g with the contralateral eye of each animal serving as a control. At 1 hours and 1, 2, 3 and 7 days (additional scoring intervals may be added to further characterize the ocular reactions), the tissues will be examined and graded for irritation and injury according to a standard scoring system of Draize *et al*[b]. After completion of the test, the scores will be analyzed, and a descriptive eye irritation rating assigned to the test article. Eye examinations may be carried out with the aid of sodium fluorescein. If deemed necessary by the study director, washed eye procedures entailing a 5 and 30 second contact period with a 5 liter wash over a 5 minute period will be conducted using 3 animals per procedure. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston-Purina, St. Louis, Missouri
[b] Draise: Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics (1965) Published by the Editorial Committee of The Association of Food and Drug Officials of the United States.

William C Mc Cormick 7/6/84
Sponsor Date

Karen D O'Malley 7/13/84
Study Director Date

Form 19171-7-A-PWO

000348

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect different significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Primary Skin Irritation Test

with T-3608

in Albino Rabbits

Experiment No.: 0984EB0368

Conducted At: Pathology and Toxicology
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: July 17, 1984 to July 20, 1984

Conducted By:

G. E. Hart 8/13/84
G. E. Hart Date
Sr. Laboratory Technician
Acute Toxicology

K. D. O'Malley 8/14/84
K. D. O'Malley, BS Date
Senior Toxicologist
Study Director

dc: K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

The results of the primary skin irritation test conducted from July 17, 1984 to July 20, 1984 at Riker Laboratories, Inc., St. Paul, Minnesota indicate that T-3608 is non-irritating (0.0/8.0) to the skin of female albino rabbits. Neither erythema nor edema were noted at any time during the study.

Introduction

. The objective of this study was to determine the primary skin irritation potential of T-3608 to the skin of female albino rabbits. This study was conducted for research and development purposes and is, therefore, not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rabbits of the New Zealand breed[a] were used in the evaluation of the primary skin irritating properties of the test article. The test procedure was modeled after that of Draize et al[b].

One day prior to the application of the test article, the hair was clipped from the back and flanks of each rabbit and two test sites selected lateral to the midline of the back approximately ten centimeters apart. One of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact.

The test article (0.5 gm) was applied to each of the test sites on each rabbit and immediately covered with two-inch square gauze patches. The patches, which were placed directly over the test sites, were secured with gauze wrap. The trunk of each animal was then wrapped with impervious plastic sheeting[c] which held the patches in position during the one day exposure period.

At the end of one day, the plastic wrappings, patches, and all residual test article were removed[d]. One hour and 48 hours after removal of the test article, the intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 - 4.

The average irritation produced was evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites were added.

[a] Hazleton Dutchland, Inc., Denver, PA
[b] Draize: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1965).
[c] 10 x 12 x .002 Extra Clear polyethylene sleeves, PPC Industries, Inc., Wheeling, Illinois.
[d] The test article was removed with water.

These two values were totaled and divided by four to obtain the mean primary irritation index. The scoring criteria for erythema and edema are shown below.

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined	1
	Pale red in color and area definable	2
	Definite red in color and area well defined.	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm.	2
	Area well defined and raised approximately 1 mm.	3
	Area raised more than 1 mm.	4
	Maximum Primary Irritation Score =	8

The following grading system was used to arrive at a descriptive primary skin irritation rating:

Mean Primary Irritation Score (Range of Values)	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reactions caused are beyond simple erythema and edema, e.g. necrosis, escharosis, hemorrhage. The results are presented in Table 1. The protocol, principal personnel involved in the study, composition characteristics and Quality Assurance statement are contained in Appendices I - IV.

Table 1

Primary Skin Irritation Test - Albino Rabbits

with T-3608

Animal Number	Irritation Scores for Abraded Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.	Irritation Scores for Intact Skin Sites after Removal: 1 Hour Er.	1 Hour Ed.	48 Hours Er.	48 Hours Ed.
4B1146	0	0	0	0	0	0	0	0
4B1149	0	0	0	0	0	0	0	0
4B1152	0	0	0	0	0	0	0	0
4B1155	0	0	0	0	0	0	0	0
4B1147	0	0	0	0	0	0	0	0
4B1150	0	0	0	0	0	0	0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Subtotal			0.0				0.0	

Rating: Non-irritating

Primary Irritation Index: 0.0/8.0

Key: Er. = Erythema
Ed. = Edema

APPENDIX I Riker Experiment No.: 0984EB0368

PROTOCOL

TEST: Acute Primary Skin Irritation Test

SPONSOR: 3M Commercial Chemicals Division

CONDUCTED BY: **Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota**

TEST ARTICLE: T-3608

CONTROL ARTICLE: N/A

PROPOSED STARTING/COMPLETION DATE OF TEST: 7/84 - 10/84

TEST SYSTEM: Female New Zealand White Albino Rabbits

SOURCE: Hazelton Dutchland
Denver Pa

OBJECTIVE: To determine the irritation potential of the test article to the skin of 6 animals. Rabbits were selected as the test system due to their historical use, sensitivity to irritants, ease of handling and general availability.

METHOD: The animals will be housed in standard wire-mesh cages in temperature and humidity controlled rooms with food[a] and water offered *ad libitum*. Each animal will be assigned a numbered ear tag, which will correspond to a card affixed to the outside of the cage. Prior to the application of the test article, the hair will be clipped from the back and flanks of each animal and 2 test sites selected lateral to the midline of the back approximately ten centimeters apart. 1 of the 2 sites will be abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site(s) will remain intact. The test article (0.5 grams) will be applied to 1 abraded and 1 intact site(s) on each animal, covered ~~with~~ and ~~and~~ secured with gauze. The trunk of each animal will then be wrapped with impervious plastic sheeting which will occlude the test article during the 1 day exposure period. One hour and 48 hours after removal of the test article, the intact and abraded test sites will be examined and scored separately for erythema and edema on a graded scale of 0 to 4[b]. The average irritation produced will be evaluated by adding the mean scores for erythema and edema of the intact test sites one and 48 hours post removal of the test article. Similarly, the mean scores for erythema and edema of the abraded test sites will be added. These two values will be totaled and divided by four to obtain the mean primary irritation index and then assigned a descriptive primary skin irritation rating as follows:

Mean Primary Irritation Score	Descriptive Rating
0	Non-irritating
0.1 - 0.5	Minimally Irritating
0.6 - 1.5	Slightly Irritating
1.6 - 3.0	Mildly Irritating
3.1 - 5.0	Moderately Irritating
5.1 - 6.5	Severely Irritating
6.6 - 8.0	Extremely Irritating

The rating for a test article may be increased if the reaction caused is beyond erythema and edema and are deemed to be of importance in the interpretation of the results. All raw data generated by the study director and the final report will be stored in the Riker Laboratories' Archive, St. Paul, Minnesota.

[a] Purina Rabbit Chow, Ralston Purina Co., St. Louis, Missouri

[b] Draize: Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics (1965) Published by the Editorial Committee of the Association of Food and Drug Officials of the United States.

William C. McCormick 7/6/84
Sponsor Date

Karen D. Malley 7/13/84
Study Director Date

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000357

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Sr. Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Senior Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Toxicology Testing
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Act of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not officially regulated by the Good Laboratory Practice Regulation of 1978, and therefore a statement signed and prepared by the Compliance Audit department is not applicable.

The standard operating procedures of this laboratory does adhere to the general principles of this regulation. The Compliance Audit department does inspect different significant phases for studies underway in the Acute Toxicology Laboratory on a recurring cycle, and the facilities are examined on a three month schedule. In addition a select number of Research & Development studies are routinely picked at random from the Archives by the Compliance Audit department for review.

Acute Oral Toxicity Screen with T-3066CoC in Albino Rats

Experiment No.: 0981AR0146

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: April 2, 1981 to May 14, 1981

Conducted By: K D O'Malley 6/5/81
K. D. O'Malley, BS Date
Advanced Toxicologist
Study Director

Reviewed By: K L Ebbens 7/13/81
K. L. Ebbens, BS Date
Supervisor, Acute Toxicology

dc: M. T. Case
K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

The acute oral toxicity screen with T-3066CoC was conducted from April 2, 1981 to May 14, 1981 at Riker Laboratories, Inc., St. Paul, Minnesota using male and female albino rats ranging in body weight from 157-289 grams. The test article was administered by gastric intubation at dosage levels of 5,000 and 500 mg/kg body weight with mortalities of 7/10 and 0/10 respectively. Untoward behavioral reactions were noted only in the 5,000 mg/kg dose group animals and consisted of hypoactivity, lethargy, prostration and diarrhea. The onset of the reactions occurred from 120 minutes to 1 day post dose and all reactions subsided by day 4 or death precluded recovery. Body weight gains were noted in all animals which survived the 14 day study period. Necropsies performed at termination of the study revealed no visible lesions among the surviving animals while hemorrhage of the gastrointestinal track or lungs were noted in all animals which died acutely. The approximate LD50 of T-3066CoC is less than 5,000 mg/kg and greater than 500 mg/kg in fasted male and female albino rats.

Introduction

The objective of this study was to approximate the acute oral LD50 of T-3066CoC in fasted male and female albino rats. This study is not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principles of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rats[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rats were housed in suspended, wire-mesh cages in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] plus water ad libitum except during the 16 - 20 hour period immediately prior to gastric intubation when food was withheld.

Groups of five male and five female rats were administered the test article at preselected dosage levels. The doses were administered at a constant volume of 10ml /kg directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubation needle[c].

After gastric administration of the test article, the rats were returned to their cages and observed for the following 14 days. Initial and final body weights, mortalities (Table 1) and adverse reactions (Table 2) were recorded. A necropsy was conducted on all animals that died during the study as well as those euthanatized at the end of the 14 day observation period (Table 1). The protocol, principal personnel involved in the study, composition characteristics, and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri
[c] Popper and Sons, Inc., New Hyde Park, New York

TABLE 1

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3066CoC

Mortality, Necropsy and Body Weight Data

Dose [a] (mg/kg)	Sex	Animal Number	Individual Body Weights (g) Test Day Number: 0	14	Number Dead / Number Tested	Percent Dead
5000	M	1R2612	246	(1 Day)	5/5	100
		1R2613	267	(1 Day)		
		1R2614	289	(4 Days)		
		1R2615	266	(1 Day)		
		1R2616	263	(1 Day)		
5000	F	1R2595	234	(2 Days)	2/5	40
		1R2596	238	(2 Days)		
		1R2597	219	252		
		1R2598	239	290		
		1R2599	232	272		
500	M	1R4113	235	320	0/5	0
		1R4114	227	319		
		1R4115	235	310		
		1R4116	228	332		
		1R4117	213	295		
500	F	1R4051	166	218	0/5	0
		1R4052	167	213		
		1R4053	160	192		
		1R4054	158	191		
		1R4055	157	205		

Note: Figures in parenthesis indicate time of death.

[a] The test article was administered as a suspension in cottonseed oil.

The acute oral LD50 is less than 5000 mg/kg and greater than 500 mg/kg in fasted male and female albino rats.

Necropsy

Necropsy of the animals which died acutely revealed hemorrhagic gastrointestinal track lungs while no visible lesions were noted upon necropsy of the animals which survived the 14 day observation period.

Table 2

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3066CoC

Summary of Reactions

Dose mg/kg	Reactions / Sex	Observation Periods: Number Affected/Number Dosed — Minutes 1-30	60	120	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
5000	M																	
	Hypoactivity	-	-	-	-	1/1	1/1	*										
	Prostration	-	-	-	1/1	0/1	-	*										
	Diarhhea	-	-	1/5	0/1	-	-	*										
5000	F																	
	Hypoactivity	-	-	-	1/5	0/3	3/3	0/3	-	-	-	-	-	-	-	-	-	-
	Lethargy	-	-	-	4/5	3/3	0/3	-	-	-	-	-	-	-	-	-	-	-
	Prostration	-	-	-	1/5	0/3	-	-	-	-	-	-	-	-	-	-	-	-
500	M																	
	No significant reaction																	
500	F																	
	No significant reaction																	

No significant reactions (-)
*Total death

APPENDIX I

PROTOCOL

TEST: Acute Oral Toxicity

SPONSOR: 3M Commercial Chemicals Division

CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota

TEST ARTICLE: T-3066L0C

CONTROL ARTICLE: N/A

PROPOSED STARTING/COMPLETION DATE OF TEST: 4/[illegible] - 7/81

TEST SYSTEM AND SOURCE: Rat, Charles River Breeding Laboratories, Wilmington, [illegible]

Sex: M,F
Number: 5,5
Weight Range: 200-300 gm

OBJECTIVE: The objective of this test will be to characterize the acute oral toxicity of the test article in albino rats. Rats were selected as a test system for reproducibility of response, historical use, ease in handling and general availability.

METHOD: The animals will be housed in stainless steel suspended wire mesh cages in temperature and humidity controlled rooms during both the quarantine and test periods, with food[a] and water offered ad libitum[b]. Each animal will be identified by color coding, according to the laboratory's standard operating procedure, which will correspond to a card affixed to the outside of the cage. A single dosage of 5,000 mg/kg will be administered each animal, however, if this dosage level does not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, the animals will be returned to their cages and observed for any untoward behavioral reactions for the following 14 days. Initial and final body weights will be recorded. A gross necropsy which will include, but not be limited to, heart, lungs, liver, kidneys and general gastrointestinal tract will be conducted on all animals which die during the conduct of the test as well as the animals surviving the test period. Any gross abnormalities which are observed during the conduct of the necropsy will be recorded with specific mention to the organ and/or site observed. The acute median lethal dose (LD50) of the test article will be calculated, if possible, using a probit analysis method at the end of the observation period. All raw data and the final report will be stored in the Riker Laboratories Archives, St. Paul, Minnesota.

[a] Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri

[b] Except during a 16-20 hour period immediately prior to dosing when food will be withheld.

William T. McCormick 3/24/81 [signature] 3/31/81

Sponsor Date Study Director Date

1. The weight limit is extended to 150 - 310 gms in order to expediate initiation of the study

ICDE Malley 5/4/81
Study Director Date

2. _____

Study Director Date

3. _____

Study Director Date

4. _____

Study Director Date

5. _____

Study Director Date

6. _____

Study Director Date

7. _____

Study Director Date

8. _____

Study Director Date

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Advanced Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore a statement signed and prepared by the Quality Assurance group is not applicable. This study was, however, audited by the Quality Assurance group.

In addition to the data audit, different significant phases for studies underway in the Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Laboratory Quality Assurance on a three month schedule.

MUTAGENICITY TEST ON

T- 6294

IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY

FINAL REPORT

AUTHOR

Hemalatha Murli, Ph.D.

PERFORMING LABORATORY

Corning Hazleton Inc. (CHV)
9200 Leesburg Pike
Vienna, Virginia 22182

LABORATORY PROJECT IDENTIFICATION

CHV Study No.: 17385-0-455

SUBMITTED TO

3M
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

STUDY COMPLETION DATE

May 10, 1996

QUALITY ASSURANCE STATEMENT

Project Title: *In Vivo* Mouse Micronucleus Assay

Project No.: 20996 Assay No.: 17385

Protocol No.: 455 Edition No.: 17

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the Standard Operating Procedures of the Quality Assurance Unit and according to the general requirements of the appropriate Good Laboratory Practice regulations. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspection/Date	Findings Reported	Auditor
Dosing/03/12/1996	03/12/1996	C. Orantes
Harvest/03/13/1996	03/13/1996	C. Orantes
Draft Report Review/05/03,06/1996	05/06/1996	C. Orantes
Final Report Review/05/10/1996	05/10/1996	C. Orantes

Quality Assurance Unit 05/10/96
Date Released

STUDY COMPLIANCE AND CERTIFICATION

The described study was conducted in compliance with the Good Laboratory Practice regulations as set forth in the Food and Drug Administration (FDA) Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978, (effective June 20, 1979) with any applicable amendments. There were no significant deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria.

All test and control results in this report are supported by an experimental data record and this record has been reviewed by the Study Director. All raw data, documentation, records, protocol and a copy of the final report generated as a result of this study will be archived in the storage facilities of Corning Hazleton Inc. for at least one year following submission of the final report to the Sponsor. After the one year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc. for an additional period of time, or sent to a storage facility designated by the Sponsor.

Submitted By:

Study Director:

Hemalatha Murli

Hemalatha Murli, Ph.D.
Mammalian Cytogenetics
Department of Genetic and Cellular Toxicology

5/10/96

Study Completion Date

TABLE OF CONTENTS

SUMMARY

Mutagenicity Test on T- 6294 in an *In Vivo* Mouse Micronucleus Assay

The objective of this *in vivo* assay was to evaluate the ability of the test article, T- 6294, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice.

In the dose selection study, the test article was suspended in acetone:corn oil (40%:60%, v:v), and dosed by oral gavage at 1000, 2000, 3000, 4000, and 5000 mg/kg. Six animals (three males and three females) were assigned to each dose group. Animals were observed for three days after dosing for toxic signs and/or mortality.

Based on the results of the dose selection study, the maximum tolerated dose was estimated as 4000 mg/kg. In the micronucleus assay, the test article was suspended in acetone:corn oil (40%:60%, v:v) and dosed by oral gavage at 1000, 2000, and 4000 mg/kg. Ten animals (five males and five females) were randomly assigned to each dose/harvest time group. Vehicle and positive control groups, euthanized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow.

The test material, T- 6294, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse bone marrow micronucleus test.

Mutagenicity Test on T- 6294 in an *in vivo* Mouse Micronucleus Assay

1.0 SPONSOR: 3M

2.0 MATERIAL (Test Article)

2.1 Client's Identification: T- 6294

2.2 Date Received: January 16, 1996

2.3 Physical Description: Wax-like amber colored solid

2.4 Genetics Assay No.: 17385

3.0 TYPE OF ASSAY: *In Vivo* Mouse Micronucleus Assay

4.0 PROTOCOL NO.: 455, Edition 17

5.0 STUDY DATES

5.1 Initiation Date: January 18, 1996

5.2 Experimental Start Date: March 6, 1996

5.3 Experimental Termination Date: April 1, 1996

6.0 SUPERVISORY PERSONNEL

6.1 Study Director: Hemalatha Murli, Ph.D.

6.2 Laboratory Supervisor: Monica Vegarra, B.S.

7.0 OBJECTIVE

The objective of this *in vivo* assay was to evaluate the ability of the test article, T- 6294, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice. This study was conducted using modifications of the procedures suggested by Heddle et al. (1983).

8.0 MATERIALS

Adult male and female mice, strain Crl:CD-1®(ICR) BR, were purchased from Charles River Laboratories, Portage, MI. This healthy, random bred strain was selected to maximize genetic heterogeneity and at the same time assure access to a common source. The protocol for this study was approved by the CHV-ACUC prior to the initiation of dosing.

Animals were housed five per cage during quarantine, and housed five at randomization. The temperature and relative humidity were maintained at 72±6°F and 55±15%, respectively, except on March 9, 1996, when the relative humidity was recorded as 38.1%. A 12-hour light/12-hour dark cycle was maintained. A commercial diet (Purina® Certified Laboratory Pellets ® # 5002) and water were available ad libitum for the duration of the study. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water was analyzed on a retrospective basis for specified microorganisms, pesticides, alkalinity, heavy metals, and halogens. Sanitized caging was used for housing the animals. Personnel handling animals or working within the animal facilities were required to wear suitable protective garments and equipment.

Animals were quarantined for seven days before being placed on study. Animals were randomly assigned to study groups and were individually weighed prior to dosing. All animals were dosed based upon the individual body weights. Animals were uniquely identified by ear tag. Dose or treatment groups were identified by cage card/label.

At the termination of the study all surviving animals were euthanized by CO_2 inhalation followed by penetration of the thorax.

9.0 SOLUBILITY AND STABILITY:

The test article, T- 6294, was supplied as a wax-like amber colored solid. The solubility of the test article was evaluated in 2% high viscosity carboxymethylcellulose (CMC) and this was not a suitable vehicle. Solubility was then evaluated in acetone:corn oil (40%:60%, v:v) and a translucent, cream-colored emulsion was obtained at a concentration of approximately 421.75 mg/ml, which formed a bilayer after a short period of time. Shaking this bilayer resulted in the re-emulsification of the mixture. Acetone:corn oil (40%:60%, v:v) was the vehicle of choice for this assay. The stability of the test material under the dosing conditions of this assay is the responsibility of the sponsor.

10.0 DOSE SELECTION STUDY

10.1 Dose Selection

Dose levels of 1000, 2000, 3000, 4000, and 5000 mg/kg were administered by oral gavage for the dose selection study.

10.2 Dosing Information

The animals used in the dose selection assay were dosed on March 6, 1996. The weight range of the animals used in the dose range finding assay was 26.6 - 34.7 and 23.1 - 26.7 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 500 mg/ml stock for the high dose (5000 mg/kg). This was prepared by adding 7.5 ml of acetone (Sigma, Lot # 2435KHXG):corn oil (Duke's corn oil, Lot # 5D1712:46), (40%:60%, v:v) to 5.0025 g of T- 6294, resulting in a translucent tan and yellow bilayer (bottom and top) that became an emulsion upon shaking with a final volume of 10.0 ml. Dilutions of this stock were prepared for the 1000, 2000, 3000 and 4000 mg/kg dose levels. All dosing stocks were placed on magnetic stir plates during the dosing procedure.

Dosing was achieved using a 10.0 ml/kg dosing volume. All animals were eight weeks and two days old at the time of dosing. An outline of the dosing scheme is found in the following table.

	DOSE GROUPS	
TREATMENT	M	F
T- 6294		
1000 mg/kg	3	3
2000 mg/kg	3	3
3000 mg/kg	3	3
4000 mg/kg	3	3
5000 mg/kg	3	3

All doses given were on an acute (one-time only) basis. A total of 30 animals was used in this assay.

10.3 Results and Interpretation

All animals were examined after dosing and daily throughout the duration of the study (three days) for toxic effects and/or mortalities. All animals appeared normal immediately after dosing.

Approximately 1 hour after dosing, all animals in all dose groups appeared hypoactive and hunched.

Approximately 24 hours after dosing, all animals in all dose groups appeared hypoactive and the 5000 mg/kg dose group also appeared hunched.

Approximately 44 hours after dosing, all animals in all dose groups appeared hypoactive and hunched. Some animals in the 1000, 2000, and 3000 mg/kg dose levels had squinted eyes and others had dyspnea. Some animals in the 4000 and 5000 mg/kg dose levels had dyspnea and all had rough haircoats. One female (# 6689) from the 5000 mg/kg dose group was found dead.

Approximately 74 hours after dosing, all animals in all dose groups appeared hypoactive and hunched. The mortality data for this assay are summarized in the following table:

Summary of Mortalities Within 3 Days in Mice Dosed Acutely with T- 6294

Observations

Treatment	Male	Female
1000 mg/kg	0/3	0/3
2000 mg/kg	0/3	0/3
3000 mg/kg	0/3	0/3
4000 mg/kg	0/3	0/3
5000 mg/kg	0/3	1/3

10.4 Conclusion

Based on these results, the maximum tolerated dose was estimated to be 4000 mg/kg.

11.0 MICRONUCLEUS STUDY

11.1 Dose Selection

Based on results from the dose selection study, dose levels of 1000, 2000, and 4000 mg/kg were selected for testing in this study.

11.2 Micronucleus Assay Dosing Information

The animals used in the micronucleus assay were dosed on March 12, 1996. Cyclophosphamide (CAS # 6055-19-2; Sigma, Lot # 44H0486), the positive control, was solubilized in sterile deionized water (Lot # 19, prepared at CHV) and was administered by oral gavage at 80.0 mg/kg. The vehicle control, acetone (Sigma, Lot # 2435KHXG):corn oil (Duke's corn oil, Lot # 5D1712:46), 40%:60%, v:v, was administered concurrently with the test article at a volume of 10.0 ml/kg. The weight range of the animals used in the micronucleus assay was 25.3 - 35.6 and 21.4 - 28.4 grams for the males and females, respectively. The dosing solutions for the assay were prepared by making a 400 mg/ml stock for the high dose (4000 mg/kg). This was prepared by adding the vehicle to the test article up to a volume of 25 ml and stirring vigorously with a spatula. A translucent tan and yellow bilayer (bottom and top) that became an emulsion upon shaking was obtained. Dilutions of this stock were prepared for the remaining dose levels. All dosing stocks were placed on magnetic stir plates during preparation and the dosing procedure. A second group of animals (designated Secondary Dose Group) was also assigned to the study and was dosed with the high dose of the test article. These animals were only used in the assay as replacements for any which died in the primary dose group.

Ten animals (five males and five females) were randomly assigned to each dose/-harvest time group. Vehicle and positive control groups, euthanized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow. An outline of the dosing scheme is found in the following table:

Dosing Scheme for Micronucleus Assay

Treatment	Number of Animals Assigned: Primary Dose Groups 24 Hr M	24 Hr F	48 Hr M	48 Hr F	72 Hr M	72 Hr F	Secondary Dose Groups[a] Male	Secondary Dose Groups[a] Female
T- 6294								
100 mg/kg	5	5	5	5	5	5	-	-
200 mg/kg	5	5	5	5	5	5	-	-
400 mg/kg	5	5	5	5	5	5	5	5
Vehicle Control, 40% Acetone/60% Corn Oil 10.0 ml/kg	5	5	-	-	-	-	-	-
Positive Control, Cyclophosphamide, 80.0 mg/kg	5	5	-	-	-	-	-	-

[a] The animals assigned to the secondary dose groups were dosed and were only used to replace animals which died in the primary dose group at the high dose level. All extra animals not used as replacements were euthanized at the completion of the trial.

The age of the animals at the time of dosing was eight weeks and one day. A total of 120 animals was used in this assay

Volumes dosed were 10.0 ml/kg based upon individual animal weights.

12.0 BONE MARROW HARVEST, SLIDE PREPARATION AND ANALYSIS

At the appropriate harvest time, the animals were euthanized with CO_2, followed by penetration of the thorax. The adhering soft tissue and epiphyses of both femora were removed. The marrow was flushed from the bone and transferred to centrifuge tubes containing 3 - 5 ml bovine serum (one tube for each animal). Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air dried. The slides were fixed in methanol, and stained in May-Grunwald solution followed by Giemsa (Schmid, 1975). The air-dried slides were coverslipped using Depex® mounting medium.

The slides were coded for analysis, and scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. Standard forms were used to record these data. One thousand PCEs per animal were scored. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The normal frequency of micronuclei in this Crl:CD-1®(ICR) BR strain is about 0.0-0.4%.

The frequency of PCEs versus NCEs was determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring the first 1000 erythrocytes.

13.0 EVALUATION CRITERIA:

13.1 General

The criteria for the identification of micronuclei were those of Schmid (1976). Micronuclei were darkly stained and generally round, although almond and ringshaped micronuclei occasionally occurred. Micronuclei had sharp borders and were generally between 1/20 and 1/5 the size of the PCE. The unit of scoring was the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-grey and red, respectively).

13.2 Data Presentation and Interpretation

Data are summarized by sex and dose groups for the different time points. Individual animal data are also presented. The analysis of these data was performed using an analysis of variance (Winer, 1971) on either untransformed (when variances are homogeneous) and rank transformed (when variances are heterogeneous) proportions of cells with micronuclei per animal. If the analysis of variance was significant ($p<0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were significantly different from the negative control. Analyses were performed separately for each harvest time and sex combination. The criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level was considered negative. In either case, the final decision was based on scientific judgment.

14.0 RESULTS AND INTERPRETATION:

All animals were observed immediately after dosing and periodically throughout the duration of the assay for toxic symptoms and/or mortalities. All animals in the vehicle and positive control groups appeared normal after dosing and remained healthy until the appropriate harvest times.

All test article dosed groups appeared normal immediately after dosing. Approximately one hour after dosing, all animals at all dose levels appeared hypoactive.

Approximately 21.5 hours after dosing, all animals in the 1000 mg/kg dose group appeared slightly hypoactive, with some showing signs of dyspnea. All animals in the 2000 mg/kg dose group appeared hypoactive, with most showing signs of dyspnea, and some females also had rough hair coats and lacrimination. All animals in the 4000 mg/kg dose group appeared hypoactive, with most showing signs of dyspnea, and most females also had ungroomed hair coats and excessive lacrimination. Three females (#'s7243, 72 hour harvest group; 7133, 7214, secondary dose group) from the 4000 mg/kg dose group were found dead.

Approximately 45.5 hours after dosing, all animals in the 1000 mg/kg dose group appeared slightly hypoactive, with some having rough hair coats. All animals in the 2000 mg/kg dose group appeared slightly hypoactive, and some females also were ungroomed and hunched. One female (#7209) from the 2000 mg/kg dose and 72 hour harvest group was found dead. All animals in the 4000 mg/kg dose group appeared slightly hypoactive, with several showing signs of dyspnea, and some females also were hunched and had ungroomed hair coats.

Approximately 69.5 hours after dosing, all animals in all dose groups appeared normal, except for one female (#7192) from the 1000 mg/kg dose group appeared hypoactive, cold to touch, had dyspnea, and was hunched.

The test article, T- 6294, induced no significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls in either sex or at any of the harvest times. The PCE/NCE ratios in the males from the positive control group, 24 and 48 hour males from the 1000 and 2000 mg/kg dose groups, and 48 hour males from the 4000 mg/kg dose group were significantly higher than the corresponding vehicle control males. The positive control, CP, induced significant increases in micronucleated PCEs in both sexes as compared to the vehicle controls, with means and standard errors of 2.42% ± 0.12% and 5.14% ± 0.65% for the males and females, respectively. The data summarized by dose group are presented in Table 1 and individual animal data are found in Tables 2 through 7. Historical control data are presented in Table 8.

15.0 CONCLUSION:

The test material, T- 6294, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse micronucleus assay.

16.0 REFERENCES:

Dunnett, C.W.: A multiple comparisons procedure for comparing several treatments with a control. J. Am. Statist. Assoc., 50:1096-1121, 1955.

Dunnett, C.W.: New tables for multiple comparisons with a control. Biometrics, 20:482-491, 1964.

Heddle, J.A., Hite, M., Kirkhart, B., Larsen, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F.: The induction of micronuclei as a measure of genotoxicity. Mutation Res., 123:61-118, 1983.

Schmid, W.: The micronucleus test. Mutation Res., 31:9-15, 1975.

Schmid, W.: The micronucleus test for cytogenetic analysis. Chemical Mutagens: Principles and Methods for Their Detection, Vol. 4 (A. Hollaender, ed.). Plenum, pp. 31-53, 1976.

Winer, B.J.: Statistical Principles in Experimental Design, McGraw-Hill, New York, Second Edition, 1971.

17.0 DEVIATION FROM THE SIGNED PROTOCOL

Due to unknown reasons, on March 9, 1996, the relative humidity was recorded as 38.1%. This had no impact on the animals or the integrity of the study.

18.0 EXPERIMENT DATA TABLES

TABLE 1

MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY: 17385

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
CONTROLS							
VEHICLE	40% Acetone/ 60% Corn oil	24 hr	0.12 ± 0.06	0.02 ± 0.02	0.07 ± 0.03	0.59 ± 0.07	0.74 ± 0.10
POSITIVE	CP 80.0 mg/kg	24 hr	2.42 ± 0.12*	5.14 ± 0.65*	3.78 ± 0.55*	0.89 ± 0.07*	0.91 ± 0.05
TEST ARTICLE	1000 mg/kg	24 hr	0.08 ± 0.06	0.04 ± 0.02	0.06 ± 0.03	1.05 ± 0.03*	0.84 ± 0.03
		48 hr	0.10 ± 0.04	0.12 ± 0.06	0.11 ± 0.03	0.92 ± 0.07*	1.00 ± 0.03
		72 hr	0.14 ± 0.04	0.30 ± 0.28	0.22 ± 0.13	0.54 ± 0.08	0.58 ± 0.05
	2000 mg/kg	24 hr	0.02 ± 0.02	0.06 ± 0.06	0.04 ± 0.03	1.02 ± 0.05*	0.82 ± 0.09
		48 hr	0.12 ± 0.04	0.04 ± 0.02	0.08 ± 0.02	0.89 ± 0.04*	1.00 ± 0.06
		72 hr	0.20 ± 0.07	0.03 ± 0.03	0.12 ± 0.05	0.48 ± 0.09	0.58 ± 0.08
	4000 mg/kg	24 hr	0.14 ± 0.07	0.12 ± 0.06	0.13 ± 0.04	0.67 ± 0.07	0.76 ± 0.05
		48 hr	0.04 ± 0.02	0.08 ± 0.06	0.06 ± 0.03	0.93 ± 0.04*	0.80 ± 0.07
		72 hr	0.10 ± 0.00	0.04 ± 0.02	0.07 ± 0.02	0.48 ± 0.07	0.55 ± 0.03

* Significantly greater than the corresponding vehicle control, $p<0.05$. CP = Cyclophosphamide

TABLE 2

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	**MALE**			
VEHICLE CONTROL	40% Acetone/60% Corn oil	7128	0	0.75
		7130	0	0.68
		7167	3	0.32
		7169	2	0.60
		7179	1	0.62
POSITIVE CONTROL	CP 80.0 mg/kg	7132	25	0.95
		7134	26	0.74
		7139	27	1.11
		7153	22	0.88
		7155	21	0.78
TEST ARTICLE	1000 mg/kg	7129	0	1.08
		7149	3	0.99
		7159	1	1.08
		7165	0	0.97
		7176	0	1.11
	2000 mg/kg	7136	0	0.89
		7162	1	1.06
		7166	0	1.10
		7170	0	1.12
		7180	0	0.91
	4000 mg/kg	7142	0	0.67
		7150	1	0.59
		7157	0	0.73
		7163	2	0.47
		7171	4	0.87

CP = Cyclophosphamide
MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 3

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	FEMALE			
VEHICLE CONTROL	40% Acetone/60% Corn oil	7190	0	0.96
		7194	0	0.63
		7200	1	0.79
		7215	0	0.90
		7222	0	0.41
POSITIVE CONTROL	CP 80.0 mg/kg	7196	52	0.98
		7201	56	1.04
		7207	36	0.84
		7208	40	0.75
		7211	73	0.92
TEST ARTICLE	1000 mg/kg	7189	1	0.80
		7193	1	0.94
		7198	0	0.85
		7237	0	0.78
		7242	0	0.86
	2000 mg/kg	7204	0	0.97
		7216	0	1.01
		7225	0	0.51
		7233	3	0.80
		7241	0	0.79
	4000 mg/kg	7206	1	0.95
		7221	0	0.71
		7229	2	0.76
		7232	0	0.71
		7235	3	0.66

CP = Cyclophosphamide
MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 4

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
48 HOUR HARVEST	MALE			
TEST ARTICLE	1000 mg/kg	7131	2	0.83
		7135	0	0.89
		7158	2	0.81
		7177	1	0.90
		7178	0	1.18
	2000 mg/kg	7137	0	0.89
		7145	2	0.96
		7151	1	0.73
		7172	1	0.89
		7182	2	0.99
	4000 mg/kg	7127	0	0.93
		7140	0	0.81
		7141	1	0.85
		7144	1	1.02
		7160	0	1.03

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 5

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
48 HOUR HARVEST	FEMALE			
TEST ARTICLE	1000 mg/kg	7186	3	0.89
		7212	2	1.10
		7217	0	1.04
		7228	0	0.98
		7236	1	0.98
	2000 mg/kg	7197	0	1.04
		7205	1	1.18
		7226	1	0.94
		7227	0	0.81
		7245	0	1.02
	4000 mg/kg	7188	1	0.63
		7210	0	0.85
		7218	3	1.03
		7220	0	0.86
		7239	0	0.66

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 6

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
72 HOUR HARVEST	MALE			
TEST ARTICLE	1000 mg/kg	7143	0	0.34
		7146	1	0.65
		7148	2	0.79
		7154	2	0.48
		7183	2	0.42
	2000 mg/kg	7138	0	0.74
		7147	1	0.47
		7156	4	0.64
		7175	2	0.30
		7184	3	0.26
	4000 mg/kg	7126	1	0.72
		7152	1	0.47
		7164	1	0.49
		7181	1	0.34
		7185	1	0.38

MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 7

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6294

ASSAY NO.: 17385

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
72 HOUR HARVEST	FEMALE			
TEST ARTICLE	1000 mg/kg	7187	0	0.56
		7192	14	0.73
		7195	0	0.47
		7213	1	0.64
		7223	0	0.52
	2000 mg/kg	7191	0	0.58
		7199	0	0.39
		7209*		
		7219	0	0.59
		7230	1	0.77
	4000 mg/kg	7202	1	0.62
		7203	0	0.56
		7224	0	0.46
		7231	0	0.51
		7234	1	0.59

* Animal found dead
MN = Micronucleus
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 8

MOUSE MICRONUCLEUS HISTORICAL CONTROL DATA 7/95 THROUGH 12/95

		% MICRONUCLEATED PCEs PER 1000 PCE MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:NCE MEAN ± S.E.	
		MALES	FEMALES	TOTAL	MALES	FEMALES
POOLED VEHICLE CONTROLS						
	MIN	0.00	0.00	0.01	0.31	0.24
	MAX	0.22	0.24	0.17	0.85	1.03
	AVG	0.087 ± 0.007	0.081 ± 0.008	0.084 ± 0.005	0.550 ± 0.021	0.587 ± 0.025
	N	47	47	47	47	47
POSITIVE CONTROLS Cyclophosphamide, 80.0 mg/kg						
	MIN	2.00	1.50	2.41	0.41	0.40
	MAX	5.68	6.36	5.38	0.72	0.79
	AVG	3.682 ± 0.240	3.170 ± 0.245	3.426 ± 0.184	0.577 ± 0.020	0.588 ± 0.026
	N	19	19	19	19	19

PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

CHV STUDY NO. _____
PROTOCOL NO. 455, EDITION 17

CORNING Hazleton

IN VIVO MOUSE MICRONUCLEUS ASSAY

Corning Hazleton Inc. (CHV) will conduct this study in compliance with Good Laboratory Practice (GLP) Regulations. This protocol, critical phase(s) of the work in progress and the final report will be subject to audit by Quality Assurance in accordance with SOPs at Corining Hazleton Inc. The study will be conducted by CHV at 9200 Leesburg Pike, Vienna, Virginia 22182.

PART 1. SPONSOR INFORMATION AND APPROVALS

I. SPONSOR IDENTIFICATION

Company Name: 3M

Address: St. Paul, MN

II. TEST ARTICLE IDENTIFICATION: T-6294

III. TEST ARTICLE ANALYSIS

Determination of the test article stability and the test article characteristics as defined in the GLP regulations is the responsibility of the Sponsor.

IV. NOTIFICATION OF REGULATORY SUBMISSION

In order to comply with the GLP regulations, consulting laboratories must be notified if all or part of a study is intended for regulatory submission. CHV maintains a master schedule of studies which fall under regulatory review. Please indicate which agency, if any, might receive the results of this study:

☑ Undetermined ☐ FDA ☐ EPA-TSCA ☐ EPA-FIFRA

☐ MAFF ☐ MOHW ☐ OECD ☐ OTHER _____

V. STUDY DATES

Proposed Experimental Start Date: _____

Proposed Experimental Termination Date: _____

VI. APPROVAL OF STUDY PROTOCOL

Study Director:

_____ Date: _____

Hemalatha Murli, Ph.D.

Sponsor's Authorized Representative:

Steven P. Gordon Date: 7/12/95

PART 2 - STUDY PROTOCOL

IN VIVO MOUSE MICRONUCLEUS ASSAY

I. OBJECTIVE

The objective of this study is to evaluate a test article for clastogenic activity and disruption of the mitotic apparatus in polychromatic erythrocyte stem cells in mouse bone marrow in vivo.

II. DEFINITIONS

Micronucleus: a small chromatin body, consisting of entire chromosome(s) and/or of acentric chromosome fragment(s), which lags behind at mitotic anaphase. After telophase, these chromosome(s) and fragment(s) may not be included in the daughter nuclei, and may form single or multiple micronuclei in the cytoplasm.

III. RATIONALE

The micronucleus test can serve as a rapid screen for clastogenic agents and test articles which interfere with normal mitotic cell division (Schmid, 1975; Heddle et al., 1983). Micronuclei are formed from chromosomes or chromosome fragments left behind during anaphase and can be scored during interphase because they persist (Schmid, 1975). In this assay, polychromatic erythrocytes (PCEs) in the bone marrow are scored for the presence of micronuclei. During maturation from erythroblast to erythrocyte the nucleus is extruded, while micronuclei, if present, remain in the cytoplasm. Detection of micronuclei in non-nucleated cells is thus facilitated, and time involved in searching for metaphase spreads in treated cell populations is eliminated. Test articles affecting spindle-fiber function or formation as well as clastogenic agents can be detected through micronucleus induction (Schmid, 1975).

IV. MATERIALS

A. Animals

Young adult male and female mice of the ICR strain, 8-10 weeks old at the time of dosing, will be purchased from Charles River Laboratories, Inc., or Harlan Sprague-Dawley, Inc. This strain has been selected to

maximize genetic heterogeneity and at the same time ensure access to a common source.

B. Control Articles

Cyclophosphamide (CP, 80 mg/kg; dosing volume of 10 ml/kg) will be used as the positive control article and will be administered by oral gavage. The vehicle control article will consist of the solvent or vehicle used for the test article and will be administered by the same route as, and concurrently with, the test article and in amounts equal to the maximum volumes administered to the experimental animals. The dosing volume will not exceed 20 ml/kg for oral gavage and IP administrations. The vehicles generally used in the assay are water, 0.5% aqueous carboxymethylcellulose solution, or corn oil.

V. EXPERIMENTAL DESIGN

A. Animal Husbandry

All applicable CHV SOPs will be followed. Animals will be isolated by sex. Animals will be housed up to seven per cage during quarantine, and will be housed up to five prior to experiment initiation. Animals are housed under the following climatic conditions: temperature, 72°F ± 6°F; humidity, 55% ± 15%; light cycle, 12 hours light/dark. A commercial diet (Purina® Certified Laboratory Chow® #5002) and tap water will be available ad libitum. The feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water is analyzed biannually on a retrospective basis for specified microorganisms, pesticides, heavy metals, alkalinity, and halogens. Animals will be quarantined for at least 7 days before being placed on study.

Animals will be assigned to study groups at random according to Coning Hazleton Standard Operating Procedures. Animals will be weighed prior to dosing. They will be dosed based upon the individual animal weights. Animals will be uniquely identified by ear tag. Treatment groups will be identified by cage label/card.

Sanitary cages will be used. Personnel handling animals or working within the animal facilities will be required to wear suitable protective garments and equipment.

B. Dose Selection

The high dose generally will be selected as 80% of the maximum tolerated dose. The high dose should produce some indication of toxicity (e.g., death, depression of ratio of PCEs to normochromatic erythrocytes (NCEs). One-half and one-quarter of this high dose will normally be used as the intermediate and low dose levels, respectively. Use of a high dose increases the likelihood that a weak clastogen will be detected, and is therefore recommended.

If no appropriate range finding data are available, a range finding study can be performed. The top dose tested in the dose rangefinding study will be 5000 mg/kg. The dose levels tested will be issued as an amendment.

DOSE RANGEFINDING STUDY

The dose rangefinding study will be conducted using five treatment groups. Each of the five groups will consist of 3 male and 3 female mice.

Group Designation and Treatment Regimens

Group No.	Number of Mice Male	Female	Route	Duration (Days)
1	3	3	PO	3
2	3	3	PO	3
3	3	3	PO	3
4	3	3	PO	3
5	3	3	PO	3

The route of administration will be oral gavage. In the event that test article characteristics preclude oral gavage, IP injection will be employed. These routes of administration have been selected because they are the most common routes of administration for this test procedure. The dosing volume will not exceed 20 ml/kg for oral gavage and IP administrations. Other routes of administration that may be used are intravenous, intramuscular, sub-cutaneous administrations or by feed. The test material will generally be solubilized in one of the following solvents: water, 0.9% saline, 0.5% aqueous carboxymethylcellulose solution, or corn oil. All animals will be dosed based upon individual body weights. Dose levels will be assigned by a protocol amendment.

Body weights will be taken prior to dosing. Dosing formulation will be prepared just prior to dosing. Dosing solutions will be prepared and held at ambient temperatures until dosing (0-2 hours). All animals will be euthanized 3 days after receiving a single dose.

The animals will be observed daily for toxic signs and mortality for the duration of the study. Animals will be euthanized by CO_2 inhalation followed by penetration of the thorax.

The daily observations of toxic symptoms and/or mortalities data will be used to estimate the Maximum Tolerated Dose (MTD). Doses will then be assigned for the subsequent cytogenetics assay.

MICRONUCLEUS STUDY

C. Dosing Schedule and Route of Administration

Normally an acute dosing regimen (single administration) will be used (see Table below). Harvest will be approximately 24, 48, 72 hours after administration of the test article, and at approximately 24 hours after administration of the control articles. A total of 110 animals will be used. Equal numbers of males and females will be used at each treatment group. An additional group of animals consisting of 3-10 males and 3-10 females may be dosed as a secondary dose group with the high dose of the test material. This group will be dosed if toxicity is expected at the high dose and the animals in this group will only be used as replacements for any which die prior to euthanasia. The use of the secondary dose group will be determined by the study director. Freshly prepared solutions will be

employed. The animals will be observed daily for toxic signs and mortality.

NUMBER OF ANIMALS USED FOR MICRONUCLEUS ASSAY

Group No.	Treatment	Harvest Times After Treatment (Males and Females) 24 Hours	48 Hours	72 Hours	Total
1	Positive Control	5 + 5	---	---	5 + 5
2	Vehicle Control	5 + 5	---	---	5 + 5
3	Low Dose	5 + 5	5 + 5	5 + 5	15 + 15
4	Medium Dose	5 + 5	5 + 5	5 + 5	15 + 15
5	High Dose	5 + 5	5 + 5	5 + 5	15 + 15
	TOTAL	25 + 25	15 + 15	15 + 15	55 + 55

The route of administration will be oral gavage. In the event that test article characteristics preclude oral gavage, IP injection will be employed. The dosing volume will not exceed 20 ml/kg. These routes of administration have been selected because they are the most common routes of administration for this test procedure. Other routes of administration that may be used are intravenous, intramuscular, sub-cutaneous administrations or by feed.

D. Extraction of Bone Marrow

Euthanasia will be with CO_2, followed by penetration of the thorax, and hind limb bones will be removed for marrow extraction. The marrow will be flushed from the bone and transferred to centrifuge tubes containing 3-5 ml bovine serum (one tube for each animal).

E. Preparation of Slides

Following centrifugation to pellet the tissue, the supernatant will be removed by aspiration and portions of the pellet will be spread on slides and air-dried.

The slides will then be fixed in methanol, stained in May-Grunwald Solution and Giemsa, and protected by mounting with coverslips. For control of bias, all slides are coded for analysis.

F. Scoring the Slides

An attempt will be made to score one-thousand PCEs per animal. The frequency of micronucleated cells will be expressed as percent micronucleated cells based on the number of PCEs analyzed. The normal background frequency of micronuclei in the ICR mouse strain is around 0.0-0.4%.

The frequency of PCEs versus mature erythrocytes (NCEs) will be determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring the first 1000 erythrocytes on the slide.

VI. DATA

The criteria for the identification of micronuclei are those of Schmid (1976). Micronuclei are darkly stained and generally round, although almond and ring-shaped micronuclei occasionally occur. Micronuclei have sharp borders and are generally between 1/20 and 1/5 the size of The PCE. The unit of scoring is the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus is counted as one micronucleated PCE, not two (or more) micronuclei.

The staining procedure permits the differentiation by color of polychromatic and normochromatic erythrocytes (bluish-grey and red, respectively).

Data Presentation

The data reported will include the number of PCEs scored, the number of micronucleated PCEs, the percentage of micronucleated PCEs, and the ratio of polychromatic to normochromatic erythrocytes for each experimental animal.

Evaluation Criteria

The criteria for a positive response is a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induces neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level is considered negative. In either case, the final decision is based upon scientific judgement.

VII. TEST INTERPRETATION

The analysis of this data will be performed using an analysis of variance (Winer, 1971) on either untransformed (when variances are homogeneous) or rank transformed (when variances are heterogeneous) proportions of cells with micronuclei per animal. If the analysis of variance is significant ($p<0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) will be used to determine which dose groups, if any, are significantly different from the negative control. Analyses will be performed separately for each harvest time and sex combination.

VIII. REFERENCES

Dunnett, C.W.: A multiple comparisons procedure for comparing several treatments with a control. J. Am. Statist. Assoc., 50:1096-1121, 1955.

Dunnett, C.W.: New tables for multiple comparisons with a control. Biometrics, 20:482-491, 1964.

Heddle, J.A., Hite, M., Kirkhart, B., Larsen, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F.: The induction of micronuclei as a measure of genotoxicity. Mutation Res., 123:61-118, 1983.

Schmid, W.: The micronucleus test. Mutation Res., 31:9-15, 1975.

Schmid, W.: The micronucleus test for cytogenetic analysis. In, Chemical Mutagens: Principles and Methods for Their Detection, Vol. 4 (A. Hollaender, ed.). Plenum, pp. 31-53, 1976.

Winer, B.J.: Statistical Principles in Experimental Design, McGraw-Hill, New York, Second Edition, 1971.

IX. REPORT FORMAT

CHV employs a standard report format for each assay design. The final report will provide the following information.

- Sponsor identification.
- Quality Assurance statement.
- Statement of GLP Compliance.
- Signature of study director.
- Test article identification and CHV Study Number. A physical description of the test article and date of receipt will be included in this section.
- Type of assay and protocol number.
- Dates of study initiation and completion.
- Study director and senior technician.
- Methods.

- Evaluation criteria.
- Interpretation of results.
- Conclusions.
- References.
- Test results presented in tabular form.

X. CHANGES OR REVISIONS

Any changes or revisions of this approved protocol will be documented, signed by the Study Director, dated, and maintained with this protocol.

XI. ANIMAL CARE AND USE STATEMENT

In the opinion of the Study Director, no alternative testing methods are appropriate, the study does not duplicate any previous work with this material, and the number and species selected are appropriate. This protocol will be reviewed by the CHV-IACUC for compliance with regulatory guidelines concerning the care and use of animals. If not in compliance, a modification will be required. Any changes or revisions of this approved protocol will be sent to the CHV-IACUC for their review.

XII. RECORDS TO BE MAINTAINED

All raw data, documentation, records, protocols, and the final report generated as a result of this study will be archived in the storage facilities of Coning Hazleton Inc. for at least one year following submission of the final report to the sponsor. After the one year period, the sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc. for an additional period of time or sent to a storage facility designated by the sponsor.

AMENDMENT TO THE STUDY PROTOCOL

STUDY TITLE: *IN VIVO* MOUSE MICRONUCLEUS ASSAY

PROTOCOL NO.: 455, Edition 17

STUDY NO.: 17385-0-455

Amendment #1

Section 2, Part V.B. The Sponsor has LD_{50} data in rats of 2459 mg/kg in males and 1580 mg/kg in females. Based on this information, the dose selection study will be conducted testing dose groups of 1000, 2000, 3000, 4000, and 5000 mg/kg. The test article will be solubilized in acetone/corn oil mixture.

STUDY DIRECTOR

Hemalatha Murli

Hemalatha Murli, Ph.D.
Mammalian Cytogenetics
Department of Genetic and Cellular Toxicology

2/28/96
Date

AMENDMENT TO THE STUDY PROTOCOL

STUDY TITLE: *IN VIVO* MOUSE MICRONUCLEUS ASSAY

PROTOCOL NO.: 455, Edition 17

STUDY NO.: 17385-0-455

Amendment #2

Section 2, Part V.C Based on the results of the dose selection study, dose levels of 1000, 2000, and 4000 mg/kg will be tested in the mouse micronucleus assay. A secondary dose group will also be used.

STUDY DIRECTOR

Hemalatha Murli

3/11/96

Hemalatha Murli, Ph.D.
Mammalian Cytogenetics
Department of Genetic and Cellular Toxicology

Date

7 MECHANISM OF TOXICITY OF A UNIQUE PESTICIDE: N-ETHYLPERFLUOROOCTANE SULFONAMIDE (NEPFOS), AND ITS METABOLITE PERFLUOROOCTANE SULFONAMIDE (PFOS) TO ISOLATED RABBIT RENAL CORTICAL MITOCHONDRIA (RCM). T J Cross and R G Schnellmann. Dept. Physiol./Pharmacol., Coll. Vet. Med., University of Georgia, Athens, GA.

NEPFOS is currently being evaluated as a pesticide for the red imported fire ant. Previous studies from this laboratory showed that an early effect of NEPFOS and PFOS on rabbit renal proximal tubules was a concentration-dependent (5-200 uM) increase in ouabain-insensitive respiration (RESP). The goal of this study was to determine whether the increased RESP resulted from uncoupling of oxidative phosphorylation (OX PHOS). NEPFOS (5-100 uM) and PFOS (0.5-50 uM) increased state 4 RESP of RCM respiring on pyruvate/malate or succinate in the absence of a phosphate acceptor or in the presence of oligomycin, an inhibitor of FOF1-ATPase. The effect of NEPFOS (200 uM), PFOS (100 uM), and the known protonophore FCCP (1uM), on proton movement by RCM was examined. Immediately on addition, PFOS and FCCP, but not NEPFOS, dissipated the proton gradient. These results show that PFOS acts as a protonophore and uncouples OX PHOS by this mechanism. The lack of proton movement by NEPFOS suggests that NEPFOS may need to be metabolized to PFOS to produce cytotoxicity and uncoupling of OX PHOS. (Supported by VMES, Univ. Georgia).

ABSTRACT FROM MARCH 1989 SOT MEETING.

3M Medical Department
Medicine
Health Physics
Industrial Hygiene
Toxicology

3M Center Bldg. 220-2E-02
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3M

Analytical and Research Properties - 3M Industrial Hygiene Laboratory

January 1993

Scope: This is a method for the determination of N-Ethyl Perfluorooctanesulfonamide in air.

Summary of Method: Air is drawn through silica gel sampling tubes. The N-Ethyl Perfluorooctanesulfonamide is adsorbed on the silica gel. The primary and backup sections of the tubes are extracted separately with methanol. The liquid is then analyzed in a gas chromatograph with flame ionization or electron capture detector.

Apparatus:

1. Silica gel sampling tubes (such as SKC #226-10 or equivalent)
   Available from: SKC, Inc.
   334 Valley View Road
   Eight Four, PA 15330

2. Sampling pumps: Battery operated pumps capable of drawing air through the sample tubes at a rate of 50-500 ml/min.

3. Gas Chromatograph (such as Hewlett-Packard 5880A or equivalent) with capillary column (Restec Stabilwax 0.53 X 30 m, 1 micrometer film capillary column) and flame ionization detector (FID) or electron capture detector (ECD).

Sampling Procedure:

Sample in the employee's breathing zone at a rate of 50-500 ml/min, depending on desired length of sampling time. The maximum volume of air sampled per tube should be 30 liters. Local conditions such as very high humidity may require a smaller volume.

The air flow through the sample tube should be as indicated by the directional arrow on the tube.

At the end of the sampling period, the tubes should be capped with the supplied caps.

Analysis:

Extraction: After sampling, the primary and backup sections of the sampling tubes can be extracted separately with 1 ml of reagent grade methanol in an autosampling vial and shaken for 30 minutes.

Analysis: The samples can be injected into the gas chromatograph (2 µl injection) and analyzed by FID or ECD using a Restec Stabilwax 0.53 mm ID X 30 m, 1 micrometer film capillary column. The GC conditions we used were:

125°C for 2 minutes
10°C/minute to 225°C
Hold for 10 minutes

Injector Temperature: 225°C
FID Temperature: 225°C

Under these conditions the retention time was 6.85 minutes.

The lower quantitation limit was ~10 micrograms with an FID. A lower quantitation limit of ~0.1 micrograms was seen with an ECD.

Recovery Study:

Fifty to 1000 micrograms of N-Ethyl Perfluorooctanesulfonamide was spiked onto the primary sections of silica gel sample tubes. Thirty liters of precleaned air was then passed through the sample tubes at 1.0 liter/minute. The samples were analyzed as described above.

The regression equation for the standard material was:

µgs analyte* = 0.0138 analyte area - 15.3 for concentrations from 25-1000 µgs (r^2 - 0.99).

* N-ethyl Perfluorooctanesulfonamide

Based on this calibration curve, the average recovery 95 ± 11%. There was no breakthrough from the primary sections to the backup sections under the conditions described above.

(SDS127-A&RPROP)

7. N-MeFOSA	N-methyl perfluorooctanesulfonamide

Acute Toxicity

1) Acute Oral Toxicity – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503499, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), July 12, 1985, with Protocol

2) Primary Dermal Irritation – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503500, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June 24, 1985, with Protocol

3) Primary Eye Irritation – Method, Summary, Pathology QAU Report, Hazleton Laboratories America, Inc., Project No. 50503501, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June 24, 1985, with Protocol

4) Acute Oral Toxicity – Method, Summary, Pathology; Primary Dermal Irritation – Method, Summary; Primary Eye Irritation – Method, Summary; QAU Report; Raw Data Appendix, Hazleton Laboratories America, Inc,. Project No. 50202473, 3M Reference No. T-3727 (F-10034, Lot 7, distilled wide-range), May 7, 1985, with Protocol

5) Acute Oral Toxicity Screen with T-3065CoC in Albino Rats, Riker Laboratories, Inc., Experiment No. 0981AR0145, May 15, 1981

Genotoxicity

1) In Vitro Microbiological Mutagenicity Assays of T-3752, SRI International, Project No. LSC-3145, 3M Reference No. T-3752 (F-7075-4, water-washed, acid washed), June, 1985

2) In Vitro Microbiological Mutagenicity Assays of T-3727, SRI International, Project No. LSC-3145, 3M Reference No. T-3727 (F-10034, Lot 7, distilled wide-range), March, 1985

HAZLETON LABORATORIES AMERICA, INC.
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

SAMPLE NUMBER: 50503499
SAMPLE ENTERED: 05/15/85
REPORT PRINTED: 07/12/85

JANINE GLEASON
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

T-3752

PURCHASE ORDER NUMBER: T357842, REL. #513

ENCLOSED: ACUTE ORAL TOXICITY - METHOD, SUMMARY, PATHOLOGY
QAU REPORT
RAW DATA APPENDIX

SIGNED: Steven M. Glaza 7-12-85
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

SAMPLE NUMBER: 50503499

T-3752

OECD ORAL SCREEN

Objective: To determine the acute oral toxicity produced when a test material is administered by oral gavage to rats according to the Organisation of Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 401, Acute Oral Toxicity, adopted May 12, 1981.

Test Material: T-3752
Physical Description: Brown granular solid
Stability of Test Material: Sponsor has purity and stability determinations on file.

Test Animal: Young adult male and female albino rats of the Sprague-Dawley strain were procured, maintained in group cages in temperature- and humidity-controlled quarters, provided continuous access to Purina Rodent Chow and water, and held for an acclimation period of at least 7 days.

Acclimated animals were chosen at random for the study. Test animals were housed by sex in groups of five and identified by animal number and corresponding ear tag. Food and water were available ad libitum throughout the study, except for an overnight period just before test material administration when food, but not water, was withheld.

Reason for Species Selection: The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Method: Five male and five female rats weighing between 195 and 234 g were used for each dosage level. The study consisted of three dosage levels (0.20, 2.0 and 5.0 g/kg).

Preparation and Administration of Test Material: For each dose level, the appropriate amount of test material was mixed with corn oil and heated and stirred on a stir plate to a uniform suspension. The suspension was cooled to room temperature prior to dosing. An individual dose was calculated for each animal based upon its fasted body weight and administered by gavage. The dose volume was 15.0 ml/kg of body weight.

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours following test material administration. The animals were observed daily thereafter for 14 days for clinical signs and twice daily for mortality.

All animals were weighed just before test material administration, at 7 days and at study termination or at death.

Pathology: At study termination surviving animals were euthanatized. Animals which died during the study or were euthanatized received a gross necropsy examination and all abnormalities were recorded.

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SAMPLE NUMBER: 50503499

T-3752

OECD ORAL SCREEN (CONTINUED)

SUMMARY

Test Animal: Albino Rats - Sprague-Dawley strain
Source: Harlan Sprague-Dawley, Madison WI
Date Animals Received: 05/22/85

Temperature and Humidity of Animal Room: 19 to 24 Degrees C.; 42 to 60% Relative Humidity

Vehicle: Corn oil
Method of Administration: Oral Gavage

Date Test Started: 05/30/85 Date Test Completed: 06/20/85

Estimated Oral LD50*: Male - Between 0.20 and 2.0 g/kg of body weight
Female - Between 0.20 and 2.0 g/kg of body weight

Mortality Summary (Number of Deaths)

Dosage Level (g/kg)	Hours 0 - 4		Days 1		2		3		4		5		6		7-14		Total		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both
0.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	0/5	0/10
2.00	0	0	4	5	1	-	-	-	-	-	-	-	-	-	-	-	5/5	5/5	10/10
5.00	0	0	4	5	1	-	-	-	-	-	-	-	-	-	-	-	5/5	5/5	10/10

	Dosage Level (g/kg)	Average Body Weights (g) Initial	Day 7	Terminal
Male	0.20	224	263	294
	2.00	212	---	---
	5.00	196	---	---
Female	0.20	201	209	219
	2.00	215	---	---
	5.00	211	---	---

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SAMPLE NUMBER: 50503499

T-3752

OECD ORAL SCREEN (CONTINUED)

Clinical Signs

	Hours			Days												
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 0.20 g/kg																	
Males																	
Appeared normal	4	3	2	0	0	0	4	5	5	5	5	5	5	5	5	5	5
Hypoactivity	0	0	2	5	5	5	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	1	2	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0
Yellow-stained abdomen	0	0	0	4	4	2	0	0	0	0	0	0	0	0	0	0	0
Red-stained face	0	0	0	5	5	1	0	0	0	0	0	0	0	0	0	0	0
Females																	
Appeared normal	4	3	4	0	0	0	4	5	5	5	5	5	5	5	5	5	5
Hypoactivity	0	0	0	3	5	5	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	1	2	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Yellow-stained abdomen	0	0	0	0	3	3	1	0	0	0	0	0	0	0	0	0	0
Red-stained face	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Dosage Level - 2.00 g/kg																	
Males																	
Appeared normal	5	1	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	1	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	4	5	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Yellow-stained anal area	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Bradypnea	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	4	1	-	-	-	-	-	-	-	-	-	-	-	-
Females																	
Appeared normal	5	3	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	2	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	1	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Prostration	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Bradypnea	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Red-stained anal area	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-

SAMPLE NUMBER: 50503499 PAGE 5

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OECD ORAL SCREEN (CONTINUED)

Clinical Signs (continued)

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 5.00 g/kg																	
Males																	
Appeared normal	5	4	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	1	1	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	0	4	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Dark-stained urogenital area	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	4	1	-	-	-	-	-	-	-	-	-	-	-	-
Females																	
Appeared normal	3	3	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	2	2	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	0	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-

SAMPLE NUMBER: 50503499 PAGE 6

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OECD ORAL SCREEN (CONTINUED)

PATHOLOGY

Dosage Level: 0.20 g/kg of body weight Date Dosed: 06/06/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C34524	M	-	14	No visible lesions.
C34518	M	-	14	No visible lesions.
C34504	M	-	14	No visible lesions.
C34510	M	-	14	No visible lesions.
C34509	M	-	14	No visible lesions.
C34465	F	-	14	No visible lesions.
C34499	F	-	14	No visible lesions.
C34466	F	-	14	No visible lesions.
C34464	F	-	14	No visible lesions.
C34473	F	-	14	Liver - hepatic anomaly at junction of median lobes, 8 x 5 mm, liver-like.

SAMPLE NUMBER: 50503499 PAGE 7

T-3752

OECD ORAL SCREEN (CONTINUED)

PATHOLOGY (continued)

Dosage Level: 2.00 g/kg of body weight Date Dosed: 06/04/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C34483	M	1	-	Red perinasal discharge; dark red, bilateral periocular discharge; perineum - moist, stained clear yellow.
C34480	M	1	-	Red perinasal discharge; perineum - moist, stained clear yellow; eye - white intraocular material.
C34481	M	1	-	Red perinasal discharge; dark red, bilateral periocular discharge; perineum - stained clear yellow.
C34477	M	1	-	Red perinasal discharge; perineum - moist, stained clear yellow;
C34512	M	2	-	Red perinasal discharge; perineum - moist with clear fluid; bilateral red ocular discharge; jejunum and ileum - contain dark brown material.
C34493	F	1	-	Perineum - stained yellow; small intestine - contains tan to yellow mucoid material.
C34463	F	1	-	Perineum - stained yellow; small intestine - contains tan to yellow mucoid material.
C34485	F	1	-	Perineum - stained yellow; jejunum - contains dark brown mucoid material.
C34469	F	1	-	Perineum - stained yellow; small intestine - contains tan to yellow mucoid material.
C34492	F	1	-	Perineum - stained yellow; small intestine - contains tan to yellow mucoid material.

SAMPLE NUMBER: 50503499 PAGE 8

T-3752

OECD ORAL SCREEN (CONTINUED)

PATHOLOGY (continued)

Dosage Level: 5.00 g/kg of body weight Date Dosed: 05/29/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C34505	M	3	-	Stomach - contains dark brown semisolid material.
C34479	M	2	-	No visible lesions.
C34513	M	2	-	Stomach - contains dark brown semisolid material.
C34503	M	2	-	Stomach - multiple dark brown areas on glandular mucosa, up to 2 x 3 mm.
C34507	M	2	-	No visible lesions.
C34500	F	2	-	No visible lesions.
C34495	F	2	-	No visible lesions.
C34484	F	2	-	Upper thoracic cavity contains dark red, clotted material.
C34498	F	2	-	No visible lesions.
C34467	F	2	-	No visible lesions.

Deviation from the protocol: During the study period the temperature of the animal room ranged from 19 to 24 degrees C. This deviation is not considered to have had an effect on the validity of the study.

References: Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, Acute Oral Toxicity, adopted May 12, 1981.

QUALITY ASSURANCE STATEMENT

Acute Oral Toxicity Study in Rats

Study No. 50503499

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
5/21-23/85	Process Audit	5/23/85
7/12/85	Report Review	7/12/85

Diana E. Skalitzky
Inspector, Quality Assurance Unit

7/12/85
Date

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3752 Vehicle CORN OIL RT No. 50503499

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 5-22-85

Fasted: Date 6-5-85 Time ~~SMC~~ ② 3:50 p.m. Tech. SMC Room No. 3

Sex: ♂

Dosage 0.20 (g/kg)									Tech.	Date 1985	Scale Used:
Dose Volume 15.0 (ml/kg)	Dose Time 10:00 a.m.										
Animal No./Ear Tag No. C3	4524	4501	4518	4504	4510	4522	4509		sam	6-6	NA
Prefasted Body Weight (g)	NA								~~sam~~	~~6-6~~ ①	~~KT~~ →
Fasted Body Weight (g)	225	227	220	230	224	219	221		sam	6-6	KTRON 15019
Actual Dose (ml)	3.4	3.4	3.3	3.5	3.4	3.3	3.3		sam	6-6	NA
Day 7 Body Weight (g)	260	*	258	274	259	*	263		sam	6-13	KTRON 15019
Day 14 Body Weight (g)	292	*	277	310	301	*	292		SMC	6-20	KTron 15019
								Doses Verified by	MP	6-6	NA

① Recording error 6-6-85 sam
② Entry error 6-6-85 SMC

Sex: ♀

Dosage 0.20 (g/kg)									Tech.	Date 1985	Scale Used:
Dose Volume 15.0 (ml/kg)	Dose Time 10:15 a.m.										
Animal No./Ear Tag No. C3	4465	4499	4466	4464	4473	4471	4494		sam	6-6	NA
Prefasted Body Weight (g)	NA										→
Fasted Body Weight (g)	200	200	200	201	205	202	200		sam	6-6	KTRON 15019
Actual Dose (ml)	3.0	3.0	3.0	3.0	3.1	3.0	3.0		sam	6-6	NA
Day 7 Body Weight (g)	212	198	210	207	220	*	*		sam	6-13	KTRON 15019
Day 14 Body Weight (g)	220	217	210	213	235	*	*		SMC MN	6-20	KTRON 15019
								Doses Verified by	MP	6-6	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6 am	6 pm	7 am	7 pm
♂ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
♀ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Technician	sam	sam	sam	SMC	SMC	SMC	SMC	SMC	MP	sam	sam	SMC	SMC	sam	SMC
Date 1985	6/6	6/7	6/7	6/8	6/8	6/9	6/9	6/10	6/10	6/11	6/11	6/12	6/12	6/13	6/13

Dose Level	8 am	8 pm	9 am	9 pm	10 am	10 pm	11 am	11 pm	12 am	12 pm	13 am	13 pm	14 am	14 pm	Total
♂ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	0/5
♀ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	0/5
Technician	SMC	SMC	CK	CK	CK	CK	MP	MP	MP	MP	SMC	SMC	SMC	NA	MN
Date 1985	6/14	6/14	6/15	6/15	6/16	6/16	6/17	6/17	6/18	6/18	6/19	6/19	6/20	NA	6/26

NA - Not Applicable
* - Dosage calculated, but not administered

Reviewed by MN Date 6-26-85

C01020

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3752 Vehicle Corn Oil RT No. 50503499

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 5-22-85

Fasted: Date 6-3-85 Time 3:00 pm Tech. CK Room No. 3

Sex ♂

Dosage 2.0 (g/kg) / Dose Volume 15.0 (ml/kg)	Dose Time 10:45 a.m.							Tech.	1985 Date	Scale Used:
Animal No./Ear Tag No. C3	4493	4478	4480	4482	4481	4477	4512	MP	6/4	NA
Prefasted Body Weight (g)	NA									→
Fasted Body Weight (g)	205	200	208	203	211	213	221	MP	6/4	KTron 15019
Actual Dose (ml)	3.1	3.0	3.1	3.0	3.2	3.2	3.3	Sam	6-4	NA
Day 7 Body Weight (g)	Dead 190	*	Dead 196	*	Dead 197	Dead 199	DEAD 194			
Day 14 Body Weight (g)	Dead 6-5-85 SMC	*	Dead 6-5-85 SMC	*	Dead 6-5-85 SMC	Dead 6-5-85 SMC	6-6-85 Sam			
							Doses Verified by	MP	6-4	NA

♀

Dosage 2.0 (g/kg) / Dose Volume 15.0 (ml/kg)	Dose Time 11:00 a.m.							Tech.	1985 Date	Scale Used:
Animal No./Ear Tag No. C3	4493	4473	4463	4485	4469	4492	4471	MP	6/4	NA
Prefasted Body Weight (g)	NA									→
Fasted Body Weight (g)	205	205	206	234	218	211	200	MP	6/4	KTron 15019
Actual Dose (ml)	3.1	3.1	3.1	3.5	3.3	3.2	3.0	Sam	6-4	NA
Day 7 Body Weight (g)	Dead 6-5-85	*	Dead 190	Dead 211	Dead 6-5-85	Dead 6-5-85	*			
Day 14 Body Weight (g)	189 MP	*	6-5-85 MP	6-5-85 SMC	201 MP	200 MP	*			
							Doses Verified by	MP	6-4	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	Study Day 1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6 am	6 pm	7 am	7 pm	8 am	8 pm	9 am	9 pm	10 am	10 pm	11 am	11 pm	12 am	12 pm	13 am	13 pm	14 am	14 pm	total
♂ 2.0	0/5	1/5	0/5	1/5	NA																							→	NA	5/5
♀ 2.0	0/5	1/5	1/5	NA	NA																							→	NA	5/5
Technician	Sam	MP	SMC	Sam	NA																							→	NA	MH
Date 1985	6/4	6/5	6/5	6/6	NA																							→	NA	6/26

NA - Not Applicable

Reviewed by MH Date 6-26-85 MH

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3752 Vehicle CORN OIL RT No. 50503499

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 5-22-85

Fasted: Date 5-29-85 Time 3:00 pm Tech. Sam Room No. 3

♂

Dosage 5.0 (g/kg)										
Dose Volume 15.0 (ml/kg)	Dose Time 11:45 a.m.							Tech.	1985 Date	Scale Used:
Animal No./Ear Tag No. C3	4505	4479	4513	4503	4507	4512	4504	Sam	5-30	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	196	197	195	196	195	196	196	Sam	5-30	KTRON 1348
Actual Dose (ml)	2.9	3.0	2.9	2.9	2.9	2.9	2.9	Sam	5-30	NA
Day 7 Body Weight (g)	Dead 6-1-85	Dead CK	Dead CK	Dead CK	Dead CK	*	*			
Day 14 Body Weight (g)	Sam 170g	5-31-85	5-31-85	5-31-85	5-31-85	*	*			
		182g	181g	175g	176g	Doses Verified by		SG	5-30	NA

♀

Dosage 5.0 (g/kg)										
Dose Volume 15.0 (ml/kg)	Dose Time 12:00 p.m.							Tech.	1985 Date	Scale Used:
Animal No./Ear Tag No. C3	4500	4495	4484	4498	4467	4485		Sam	5-30	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	208	215	210	210	213	230		Sam	5-30	KTRON 1348
Actual Dose (ml)	3.1	3.2	3.2	3.2	3.2	3.5		Sam	5-30	NA
Day 7 Body Weight (g)	Dead CK	Dead CK	Dead CK	Dead CK	Dead CK	*				
Day 14 Body Weight (g)	5-31-85	5-31-85	5-31-85	5-31-85	5-31-85	*				
	183g	200g	199g	194g	211g	Doses Verified by		SG	5-30	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours	Study Day																												Total
	0 - 4	1		2		3		4		5		6		7		8		9		10		11		12		13		14		
		am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	
5.0 g/kg ♂	5/5	5/5	0/5	0/5	NA																							>	NA	5/5
5.0 g/kg ♀	5/5	5/5	0/5	NA	NA																							>	NA	5/5
Technician	Sam	CK	CK	Sam	NA																							→	NA	MH
Date 1985	5/30	5/31	5/31	6/1	NA																							→	NA	6/26

NA - Not Applicable

* - Dosage calculated but not administered

Reviewed by MH Date 6-26-85

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4524

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red Stained face	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Yellow Stained abdomen	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea	—	—	—	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
hypoactive	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	SMC	SMC	SMC	Sam	SMC	SMC	SMC	CK	CK	MP	MP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01023

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4518

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/5	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	—	✓	✓	✓	NE ①/②	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face	—	—	—	✓	✓	NE ②	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
hypoactive	—	—	—	✓	✓	✓	NE ③	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	SMC	SMC	SMC	Sam	SMC	SMC	SMC	CK	CK	KD	KD	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight
① Entry error 6-8-85 SMC
② Entry error 6-9-85 SMC
③ Entry error 6-10-85 SMC
NE - Not Evident
NA - Not Applicable

C01024

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4504

STUDY DAY	HOURS 1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/5	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hypoactive	—	—	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red Stained face	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained abdomen	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea	—	—	—	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	SMC	SMC	SMC	[illegible]	SMC	SMC	SMC	CK	CK	AP	AP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01025

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♂

DOSAGE LEVEL: 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4510

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
hyperactive	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained abdomen	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea	—	—	—	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	SMC	SMC	SMC	[illegible]	SMC	SMC	SMC	CK	CK	KP	KP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NK - Not Evident
NA - Not Applicable

G01026

STUDY TITLE: Acute Oral Toxicity

NT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4509

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive	—	—	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained abdomen	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	CK	CK	[illegible]	[illegible]	[illegible]	[illegible]
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01322

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀

DOSAGE LEVEL: 0.20 g/kg

ANIMAL/EAR TAG NO.: C3-4465

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	NE	NE	✓	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	✓	✓	NE	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
hypoactive	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red Stained face	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sma	Sma	Sma	Sma	SMC	SMC	SMC	Sma	SMC	SMC	SMC	CK	CK	CK	CK	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀

DOSAGE LEVEL: 0.20 g/kg

ANIMAL/EAR TAG NO.: C3-4499

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	✓	NG	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
hypoactive	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Yellow stained abdomen	—	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	SMC	SMC	SMC	Sam	SMC	SMC	SMC	CK	CK	CK	CK	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C-01029

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752

SEX ♀

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C3-4466

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	—	—	—	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive	—	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Yellow stained abdomen	—	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sman	Sman	Sman	Sman	SMC	SMC	SMC	Sman	SMC	SMC	SMC	CK	CK	KP	KP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

BLUE OUT FOR MICROFILM

C01050

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♀

DOSAGE LEVEL 0.20g/kg

ANIMAL/EAR TAG NO. C3-4464

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive	—	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	SMC	SMC	SMC	Sam	SMC	SMC	SMC	CK	CK	MP	MP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01031

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♀

DOSAGE LEVEL 0.20g/kg

ANIMAL/EAR TAG NO. C3-4473

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea	—	—	—	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
hypoactive	—	—	—	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Yellow stained abdomen	—	—	—	—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	SMC	SMC	SMC	Sam	SMC	SMC	SMC	CK	CK	MP	MP	SMC	SMC
DATE 1985	6/6	6/6	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18	6/19	6/20

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01032

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4483

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	WE	WE	NA													
Hypoactive	—	✓	✓	↓													
Diarrhea	—	✓	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01033

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752 SEX ♂

DOSAGE LEVEL 2.0g/Kg ANIMAL/EAR TAG NO. C3-4480

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	✓	NE	NA													
Hypoactive	—	—	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	Sam	Sam	Sam	MP													
DATE 1985	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01034

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4481...

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	NE	NE	NA													
Hyperactive	—	✓	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	mm	mm	mm	M													
DATE 1985.	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01035

STUDY TITLE: Acute Oral Toxicity

HT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4477

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	NE	NE	NA													
Hypoactive	—	✓	✓	↓													
DEATH	5pm	—	—	✓													
TECHNICIAN ①	4pm	5pm	4pm	NA													
DATE 1985	6/4	6/4	6/4	6/5													

① Recording error 6-4-85 5pm

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C-041036

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♂

DOSAGE LEVEL: 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4512

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	√	NE	NE	NE	X②												
Hypoactive	—	√	√	√													
Bradypnea	—	—	—	√													
Ataxia	—	—	—	√													
Red Stained around Face	—	—	—	√													
Yellow Stained Anal Area	—	—	—	√													
DEATH				X②	√												
TECHNICIAN	Sam	Sam	Sam	Sam	Sam												
DATE 1985	6/4	6/4	6/4	6/5	6/6												

√ - Sign Present
sl - Sign Present, Slight
① recording error MD 6-5-85
② error, terrors MH 6-24-85

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX ♀

DOSAGE LEVEL: 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4493

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	✓	NE	NE	NA												
Hypoactive	—	—	✓	X①	↓												
DEATH	—	—	—	✓													
TECHNICIAN	Sam	Sam	Sam	M													
DATE 1985	6/4	6/4	6/4	6/5													

① Recording error 6-4-85 Sam

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01038

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL. T-3752

SEX ♀

DOSAGE LEVEL. 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4463

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	✓	NE	NA													
Appoactive	—	—	✓	↓													
Diarrhea	—	—	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01039

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀

DOSAGE LEVEL: 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4485

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	NE	NE	NE													
Hypoactive	–	✓	✓	✓													
Diarrhea	–	–	✓	NE													
Prostrate	–	–	–	✓													
Bradypnea	–	–	–	✓													
Red Stained Face	–	–	–	✓													
Red Stained Anal Area	–	–	–	✓													
DEATH				①													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① Animal found dead in p.m. 6-5-85 JMC

C01040

STUDY TITLE: Acute Oral Toxicity

HT NO. 50503499

TEST MATERIAL: T-3752

SEX ♀

DOSAGE LEVEL: 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4469

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	✓	NE	NA													
Hypoactive	—	—	✓	↓													
Diarrhea	—	—	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL T-3752

SEX ♀

DOSAGE LEVEL 2.0g/Kg

ANIMAL/EAR TAG NO. C3-4492

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	6/4	6/4	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	6/14	6/15	6/16	6/17	6/18
APPEARED NORMAL	✓	NE	NE	NA													
Hypoactive	–	✓	✓	↓													
Diarrhea	–	✓	✓	↓													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	NA													
DATE 1985.	6/4	6/4	6/4	6/5													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01042

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♂

DOSAGE LEVEL 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4505

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	NE	NE	NA												
Hypoactive	—	—	✓	✓	NA												
Red Stained face	—	—	—	✓	NA												
Dark Stained urogenital area	—	—	—	✓	NA												
diarrhea	—	—	—	✓	NA												
DEATH					✓												
TECHNICIAN	[illegible]	[illegible]	[illegible]	CK	[illegible]												
DATE 1985	5/30	5/30	5/30	5/31	6/1												

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL. T-3752

SEX ♂

DOSAGE LEVEL. 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4479

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	NS	NA													
Hypoactive	—	—	✓	NA													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	CR													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01044

STUDY TITLE: Acute Oral Toxicity

NT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♂

DOSAGE LEVEL: 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4513

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	WNL	NA													
Hypoactive	—	—	✓	NA													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	CK													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NK - Not Evident
NA - Not Applicable

C01045

STUDY TITLE: Acute Oral Toxicity

NT NO. 50503499

TEST MATERIAL. T-3752

SEX ♂

DOSAGE LEVEL. 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4503

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	NE	NA													
Hypoactive	—	—	✓	NA													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	CR													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01046

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀ ♂ ①

DOSAGE LEVEL: 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4507

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	NE	NE	NA													
Diarrhea	—	✓	✓	NM													
DEATH	—	—	—	✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	CR													
DATE 1985	5/30	6/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight
① Recording error 5-30-85 sm

NE - Not Evident
NA - Not Applicable

G01047

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752 SEX ♀

DOSAGE LEVEL 5.0 g/kg ANIMAL/EAR TAG NO. C3-4500

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	NE	NA													
Hypoactive	—	—	✓	NA													
DEATH				✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	CK													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01048

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀

DOSAGE LEVEL: 5.0 g/kg

ANIMAL/EAR TAG NO.: C3-4495

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	✓	✓	NE	NA													
Hypoactive	—	—	✓	NA													
DEATH	—	—	—	✓													
TECHNICIAN	Smm	Smm	Smm	KK													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NK - Not Evident
NA - Not Applicable

C010449

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL T-3752

SEX ♀

DOSAGE LEVEL 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4484

	HOURS																	
STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	
APPEARED NORMAL	NE	NE	NE	NA														
Diarrhea	✓	✓	✓	NA														
Hypoactive	—	—	✓	NA														
DEATH	—	—	—	✓														
TECHNICIAN	[illegible]	[illegible]	[illegible]	CK														
DATE 1985	5/30	5/30	5/30	5/31														

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01050

STUDY TITLE: Acute Oral Toxicity

BT NO. 50503499

TEST MATERIAL: T-3752

SEX: ♀

DOSAGE LEVEL: 5.0 g/kg

ANIMAL/EAR TAG NO.: C3-4498

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13
APPEARED NORMAL	NO	NO	NO	NA													
Diarrhea	✓	✓	✓	NA													
Hyperactive	—	—	✓	NA													
DEATH	—	—	—	✓													
TECHNICIAN	Smm	Smm	Smm	CR													
DATE 1985	5/30	5/30	5/30	5/31													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01051

STUDY TITLE: Acute Oral Toxicity

RT NO. 50503499

TEST MATERIAL. T-3752

SEX ♀

DOSAGE LEVEL. 5.0 g/kg

ANIMAL/EAR TAG NO. C3-4467

	HOURS																	
STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SCHEDULED DATE	5/30	5/30	5/30	5/31	6/1	6/2	6/3	6/4	6/5	6/6	6/7	6/8	6/9	6/10	6/11	6/12	6/13	
APPEARED NORMAL	✓	✓	NE	NA														
Hypoactive	—	—	✓	NA														
DEATH	—	—	—	✓														
TECHNICIAN	[illegible]	[illegible]	[illegible]	CK														
DATE 1985	5/30	5/30	5/30	5/31														

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01052

HAZLETON LABORATORIES AMERICA, INC.

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP2069

Acute Oral Toxicity Study in Rats (OECD Guidelines)

Study No. 50503499

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

May 21, 1985

PROTOCOL TP2069

Acute Oral Toxicity Study in Rats (OECD Guidelines)

Study No.:	50503499
Study Location:	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material:	T-3752
Sponsor's Representative:	Janine Gleason
Study Director:	Steven M. Glaza
Proposed Timetable	
Starting Date:	Week of May 27, 1985
Completion Date:	Week of June 17, 1985
Final Report Date:	Week of July 15, 1985

OBJECTIVES

To determine the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, adopted May 12, 1981[1] and Principles of Good Laboratory Practice.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Test Material:	T-3752.
Physical Description:	Brown granular solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be returned to the Sponsor 30 days after issuance of the final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

TEST SYSTEM

Animal Model

Young adult male and female albino rats (approximately 7 weeks of age) of the Sprague-Dawley strain will be obtained from Harlan Sprague-Dawley, Madison, Wisconsin. Rats will be selected at random from healthy animals that had been acclimated at HLA for at least 1 week. An adequate number of extras will be purchased in order that no animal in obviously poor health is placed on test. The weight variation in animals used on test will not exceed ±20% of the mean weight (i.e., mean = 250 g, range = 200 to 300 g).

Reason for Species Selection

The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Identification

Each animal will be assigned an individual animal number and ear tag which will accompany data collected from that animal throughout the study (OP-GENB 24).

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 22°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be individually housed in screen-bottom stainless steel cages held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Purina Rat Chow®. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

PROCEDURES

Experimental Design

Initially, a single dose of 5.0 g/kg will be administered to 10 animals (five males and five females). If no test material-related mortality is produced at this level, no further testing is required. If any mortality occurs at the 5.0-g/kg dose level, at the Sponsor's request, three or four geometrically spaced dose levels may be added. Each dose level will consist of 10 animals (five males and five females). Animals will be assigned to groups according to HLA Standard Operating Procedure OP-TOX 42.

Test Material Preparation and Administration

The test material will be suspended in an appropriate vehicle. Individual dosages will be calculated based upon the animal's body weight taken just before administration of the test material and administered by gavage.

Justification of Route of Administration

This is the method for administering a known quantity of test substance and has been the route of choice historically.

Observations

The animals will be observed individually for clinical signs and mortality at 1.0, 2.5, and 4 hours after test material administration. The animals will be observed daily thereafter for at least 14 days for clinical signs and twice daily (morning and afternoon) for mortality. The duration of observations may be extended when considered necessary. The time of death will be recorded as precisely as possible.

Individual body weights will be recorded just prior to study initiation and at 7 and 14 days following test material administration and at death. Changes in body weight will be calculated and recorded when survival exceeds 1 day.

Pathology

All test animals, whether dying during the study or sacrificed at termination, will be subjected to a gross necropsy examination and abnormalities recorded.

Report

The final report will contain a description of the test material, a description of how the study was conducted, response data for clinical signs, mortality and body weights by sex, a discussion of the data, and gross pathology findings.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, Acute Oral Toxicity, adopted May 21, 1981.

2. Organisation for Economic Cooperation and Development's Principles for Good Laboratory Practive, Annex 2, 1981.

3. DHEW Publications No. (NIH) 78-23 (1978).

PROTOCOL APPROVAL

Janine Gleason — 5/24/85

Janine Gleason
Sponsor's Representative
3M

Date

Steven M. Glaza — 5-22-85

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

Date

(1107S/tji)

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

JANINE GLEASON
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 50503500
SAMPLE ENTERED: 05/15/85
REPORT PRINTED: 06/21/85

SAMPLE: T-3752

PURCHASE ORDER NUMBER: T357842, REL. #513

ENCLOSED: PRIMARY DERMAL IRRITATION - METHOD, SUMMARY
QAU REPORT
RAW DATA APPENDIX

SIGNED: Steven M. Glaza ..... 6-24-85 .....
STEVEN M. GLAZA DATE
STUDY DIRECTOR
ACUTE TOXICOLOGY

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

HAZLETON LABORATORIES AMERICA, INC.
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

SAMPLE NUMBER: 50503500

SAMPLE: T-3752

OECD SKIN IRRITATION

Objective: To determine the relative level of primary skin irritation of a test material on rabbits under semioccluded conditions according to the Organisation of Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 404, Acute Dermal Irritation/Corrosion, adopted May 12, 1981.

Test Material: T-3752
Physical Description: Brown granular solid
Purity and Stability: Sponsor has purity and stability determinations on file.

Test Animal: Young adult rabbits (approximately 14 weeks of age) of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided continuous access to Purina High Fiber Rabbit Chow and water, and held for an acclimation period of at least 7 days.

Three acclimated animals, weighing from 2212 to 2323 g, were chosen at random for the test, treated, and maintained during the observation period as specified for the acclimation period. Test animals were identified by animal number and corresponding ear tag. Approximately twenty-four hours before treatment the hair was clipped from the back of each animal.

Reason for Species Selection: Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

Preparation and Administration of Test Material: The sample was dosed as received.

Treatment: The test material was applied to the intact skin of each rabbit in the amount of 0.5 g per site and moistened with 0.9% saline. The treated area was covered with a 2.5 x 2.5-cm gauze patch secured with paper tape and overwrapped with Saran Wrap and Elastoplast tape to provide a semiocclusive dressing. Collars were applied to restrain the test animals for the 4-hour exposure period.

SAMPLE NUMBER: 50503500 PAGE 3

SAMPLE: T-3752

OECD SKIN IRRITATION (CONTINUED)

Observations: After the exposure period, the patches were removed. The test sites were washed using lukewarm tap water and disposable paper towels. The test material was removed from the test sites as thoroughly as possible without irritating the skin. Thirty minutes following removal of the test material, the degree of erythema and edema was read according to the Draize* technique. Subsequent examinations were made at 24, 48 and 72 hours after patch removal.

Individual body weights were taken just prior to study initiation.

Pathology: At study termination all animals were euthanatized and discarded.

*Draize, J. H., "Appraisal of The Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

SAMPLE NUMBER: 50503500 PAGE 4

SAMPLE: T-3752

OECD SKIN IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 05/21/85

Temperature and Humidity of Animal Room: 21 - 23 Degrees C.;
46 - 64% Relative Humidity

Date Test Started: 05/29/85 Date Test Completed: 06/01/85

Vehicle: Moistened with 0.9% saline

Individual Dermal Irritation Scores
Test Material: T-3752

Animal Number	Erythema Score Hours 4	24	48	72	Edema Score Hours 4	24	48	72
F08693	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F08704	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F08690	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Primary Dermal Irritation Scores

Observation Period	3 Rabbit Mean
4 Hours:	0.0
24 Hours:	0.0
48 Hours:	0.0
72 Hours:	0.0

Results:

No dermal irritation was observed at any time during the study period.

Deviation from the protocol: The test material was moistened with 0.9% saline rather than deionized water as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

001064

SAMPLE NUMBER: 50503500 PAGE 5

SAMPLE: T-3752

OECD SKIN IRRITATION (CONTINUED)

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 404, Acute Dermal Irritation/ Corrosion, adopted May 12, 1981.

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

QUALITY ASSURANCE STATEMENT

Primary Dermal Irritation Study in Rabbits

Study No. 50503500

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 21 CFR 58.35 (b) (6) (7). It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study and that the reported data accurately reflect the raw data of the laboratory study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
5/21-23/85	Process Audit	5/23/85
6/10/85	Report Review	6/10/85

Diana E. Skalitzky

Diana E. Skalitzky
Inspector, Quality Assurance Unit

6/12/85
Date

PRIMARY SKIN IRRITATION SCORING SCALE

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

PRIMARY DERMAL IRRITATION STUDY

Test Compound: T-3752 HLA Number: 50503500

Dose: 0.5g/site Vehicle: ① 0% moistened with 0.9% saline pH Result: NA

Date Animals Received: 5-21-85 Source: Hazleton Research Products Room Number: 161-3

Date Animals Clipped: 5-28-85 Tech: CK Initiated by: CK Date: 5/29/85

Skin Preparation: Intact Reviewed by: pgr Date: 5/29/85

Animal Number/Sex FO		86930♂	87040♂	86909♀				Technician	Recorded by	1985 Date	Ktron Scale used:
Initial Body Weight (g)		2226	2323	2212				CK	CK	5-29	15019
Observation Period											Dermal Irritation Score
4 Hours	Erythema	0	0	0							
	Edema	0	0	0				CK	CK	5-29	0.0 slh
24 Hours	Erythema	0	0	0							
	Edema	0	0	0				CK	CK	5-30	0.0 slh
48 Hours	Erythema	0	0	0						5-31 ②	
	Edema	0	0	0				Sam	Sam	~~5-30~~	0.0 slh
72 Hours	Erythema	0	0	0							
	Edema	0	0	0				Sam	Sam	6-1	0.0 slh
96 Hours	Erythema										
	Edema										
7 Days	Erythema										
	Edema										
7 Day Body Weight (g)											Scale used:

NA - Not applicable.
A - Subcutaneous hemorrhage.
B - Blanching.
N - Possible necrotic area.

Reviewed by: slh Date: 6-3-85

(42?.M)

① Entry Error CK 5-29-85
② Recording error Sam 5-31-85

C01068

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3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP2071

Primary Dermal Irritation Study in Rabbits (OECD Guidelines)

Study No. 50503500

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

May 21, 1985

PROTOCOL TP2071

Primary Dermal Irritation Study in Rabbits
(OECD Guidelines)

Study No.	50503500
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	T-3752
Sponsor's Representative	Janine Gleason
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of May 27, 1985
Completion Date	Week of May 27, 1985
Final Report Date	Week of June 24, 1985

OBJECTIVE

The objective of this study is to determine the relative level of primary skin irritation of a test material on rabbits under semioccluded conditions. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 404, Acute Dermal Irritation/Corrosion, Adopted May 12, 1981[1] and the Principles of Good Laboratory Practice.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Test Material:	T-3752.
Physical Description:	Brown granular solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be returned to the Sponsor 30 days after issuance of the final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

TEST SYSTEM

Test Animal

Young adult albino rabbits of either sex of the New Zealand White strain, approximately 14 weeks of age, will be obtained from Hazleton Research

Products Inc., Denver, Pennsylvania. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

Acclimation

Upon receipt, the animals will be taken to a designated animal room where they will be acclimated for at least 1 week before being placed on test (OP-GENB 36). During acclimation, the animals will be examined for clinical abnormalities indicative of health problems (e.g., diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, evidence of injury, etc.). Any animals regarded as unsuitable for study purposes because of poor physical condition will not be released from acclimation and the reason(s) will be documented.

Identification

Each animal in the study will be assigned a permanent identification number and will be identified with a metal ear tag (OP-GENB 24). All data collected from an animal will be recorded and filed under its identification number.

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 21°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Purina High Fiber Rabbit Chow. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

Study Design

Three rabbits will be selected at random based upon health and a body weight of 2.0-3.5 kg. Each animal will serve as its own control.

PROCEDURES

Preparation and Administration of Test Material

Twenty-four hours prior to test material administration, the hair will be clipped from the back and flanks of each animal. The treatment sites will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals.

The test material will be applied to the test area (approximately 6 cm^2) on each rabbit, in the amount of 0.5 g and will be moistened with deionized water. The treated area will be covered with a 2.5-cm x 2.5-cm gauze patch

secured with paper tape and loosely overwrapped with Saran Wrap and Elastoplast tape to provide a semiocclusive dressing. Collars will be used to restrain the animals during the 4-hour exposure period.

Reason for Route of Administration

Historically, the route of choice based on the method of Draize.[4]

Observations

After the 4 hours of exposure the patches and the test material will be removed as thoroughly as possible using water or an appropriate solvent without irritating the skin. Thirty minutes after removing the patches, the degree of erythema and edema will be recorded according to the Draize Technique (Attachment 1). Subsequent readings will be taken at 24, 48, and 72 hours after patch removal. Further observations may be recorded, as necessary, to establish reversibility. If irritation is increasing in severity at the 72-hour examination period, observations will be repeated at 96 hours and at 7 and 14 days, if applicable.

Body weights will be taken just prior to test material administration and at weekly intervals during the study. Observations and body weights will be recorded in the study notebook.

Pathology

All animals, whether dying on test or sacrificed at study termination, will be discarded.

Report

The final report will present a description of the test material, a description of the test system, dates of study initiation and termination, a tabulation of irritation data, and a description of any toxic effects other than dermal irritation.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. "Acute Dermal Irritation/Corrosion", OECD Guidelines for Testing Chemicals, Section 404, May 12, 1981.

2. Organisation for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, 1981.

3. DHEW Publications No. (NIH) 78-23 (1978).

4. Draize, J. H., "Dermal Toxicity," Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

PROTOCOL APPROVAL

Janine Gleason — 5/24/85

Janine Gleason
Sponsor's Representative
3M

Date

Steven M. Glaza — 5-22-85

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

Date

(1108S/tji)

ATTACHMENT I

PRIMARY SKIN IRRITATION SCORING SCALE

1. Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

2. Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

HAZLETON LABORATORIES AMERICA, INC.
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

JANINE GLEASON
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 50503501
SAMPLE ENTERED: 05/15/85
REPORT PRINTED: 06/21/85

SAMPLE: T-3752

PURCHASE ORDER NUMBER: T357842, REL. #513

ENCLOSED: PRIMARY EYE IRRITATION - METHOD, SUMMARY
QAU REPORT
RAW DATA APPENDIX

SIGNED: Steven M. Glaza ..... 6-24-85 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

SAMPLE NUMBER: 50503501

SAMPLE: T-3752

OECD EYE IRRITATION

Objective: To determine the level of ocular irritation produced following a single exposure of a test substance to one eye of albino rabbits according to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/ Corrosion, adopted May 12, 1981.

Test Material: T-3752
Physical Description: Brown granular solid
Purity and Stability: Sponsor has purity and stability determinations on file.

Test animal: Young adult rabbits (approximately 14 weeks of age) of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided continuous access to Purina High Fiber Rabbit Chow and water, and held for an acclimation period of at least 7 days.

Three acclimated animals, weighing from 2333 to 2504 g, were chosen at random for the test. The animals' eyes were examined within 24 hours prior to test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used. Test animals were identified by animal number and corresponding ear tag.

Reason for Species Selection: The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Preparation and Administration of Test Material: The sample was dosed as received. A bulk density determination was made to determine the weight equivalent of a 0.1 ml dose. Because individual doses should not exceed 0.10 g and the weight equivalent of 0.1 ml was 0.12 g, an individual dose of 0.1 g was weighed out for each animal.

Treatment: Each rabbit received 0.1 g of the test material placed on the everted lower lid of one eye, with the contralateral eye serving as the untreated control. The upper and lower lids were gently held together for one second to prevent loss of material and then released. The eyes of the rabbits remained unflushed.

SAMPLE NUMBER: 50503501 PAGE 3

SAMPLE: T-3752

OECD EYE IRRITATION (CONTINUED)

Observations: The treated eyes were observed for ocular irritation at 1, 24, 48, and 72 hours after treatment.

At the 72-hour reading, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize* technique.

Animals were weighed just prior to test material administration.

Pathology: At study termination all animals were euthanatized and discarded.

*Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 49-51 (1959).

SAMPLE NUMBER: 50503501

SAMPLE: T-3752

OECD EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 05/21/85

Temperature and Humidity of Animal Room: 21 to 23 Degrees C.;
46 to 64% Relative Humidity

Test Material: T-3752

Date Test Started: 05/29/85 Date Test Completed: 06/01/85

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	3 Rabbit Mean 0.1 g (Unwashed)
1 Hour:	10.7
24 Hours:	0.0
48 Hours:	0.0
72 Hours:	0.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (3) at each observation period.

Comments: No pain response (vocalization) was elicited from any animal following instillation of the test material.

No corneal irritation was observed during the study.

SAMPLE NUMBER: 50503501 PAGE 5

SAMPLE: T-3752

OECD EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F08711	1 Hour	0	0	0	1	5	1	2	0	6
	24 Hours	0	0	0	0	0	0	0	0	0
	48 Hours	0	0	0	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0	0	0	0
F08705	1 Hour	0	0	0	0	0	1	1	0	4
	24 Hours	0	0	0	0	0	0	0	0	0
	48 Hours	0	0	0	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0	0	0	0
F08721	1 Hour	0	0	0	1	5	1	2	3	12
	24 Hours	0	0	0	0	0	0	0	0	0
	48 Hours	0	0	0	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0	0	0	0

Table 2
Sodium Fluorescein Examination

Animal Number	Observation Period	
	Pre-initiation	72 Hours
F08711	NEG	NEG
F08705	NEG	NEG
F08721	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/Corrosion, adopted May 12, 1981.

2. Draize J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the United States, Topeka, Kansas, pp. 49-51 (1959).

QUALITY ASSURANCE STATEMENT

Primary Eye Irritation Study in Rabbits

Study No. 50503501

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 21 CFR 58.35 (b) (6) (7). It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study and that the reported data accurately reflect the raw data of the laboratory study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
5/21-23/85	Process Audit	5/23/85
6/10/85	Report Review	6/10/85

Diana E. Skalitzky

Diana E. Skalitzky
Inspector, Quality Assurance Unit

6/12/85
Date

PROTOCOL - ATTACHMENT 1

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Total Maximum = 10

(3) Conjunctiva

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Primary Eye Irritation Test

Initial Sodium Fluorescein Exam and Animal Body Weights

Test Compound T-3752 RT No. 50503501

pH Result NA

Dose (g) 0.1g/Eye Room No. 161-3

Dose (ml) NA Dosed By CK Date 5/29/85

Date Animals Received 5-21-85 Reviewed By pgr Date 5/29/85

Source: Hazleton Research Products

Animal No.	Sex	Initial SF*	Vocalization Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
FO-8711	♂	NEG	N	2333			
8705	♂	NEG	N	2427			
8721	♀	NEG	N	2504			
TECHNICIAN	CK	Sam	CK	CK			
RECORDED BY	CK	Sam	CK	CK			
DATE 1985	5/29	5-28	5-29	5-29			
SCALE USED			Ktron	15019			

* Sodium Fluorescein Examination

NEG = Negative

POS = Positive

NA = Not Applicable

Y = Yes

N = No

Time of Dosing: 10:45 AM CK 5-29-85

Time of first observation: 11:45 AM CK 5-29-85

Primary Eye Irritation Test Observations

Test Compound T-3752 RT No. 50503501

Test Eye Right Group N/A

N/A Washed N/A Seconds following instillation of test material, the test eye was washed with N/A ml of N/A for N/A seconds ✓ Unwashed

OBSERVATION PERIOD: 1 hour

Animal No./ Ear Tag No. FO-	8711	8705	8721			
Location of Corneal Lesions Tail <----------> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	1 INJ	0	1 INJ			
Conjunctivae -						
Redness	1	1	1			
Chemosis	2	1	2			
Discharge	0	0	3 A			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	CK	CK	CK			
Recorded By	CK	CK	CK			
Date 1985	5/29	5/29	5/29			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: slh Date: 10-3-85 Eye Irritation Score: 10.7 slh MW
(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3752 RT No. 50503501

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 24 hours

Animal No./ Ear Tag No. FO-	8711	8705	8721			
Location of Corneal Lesions Tail <----------> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	MP	MP	MP			
Recorded By	MP	MP	MP			
Date 1985	5/30	5/30	5/30			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: SLH Date: 6-3-85 Eye Irritation Score: D.O SLH

(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3752 RT No. 50503501

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 48 hours

Animal No./ Ear Tag No. FO-	8711	8705	8721			
Location of Corneal Lesions Tail <----------> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	CK	CK	CK			
Recorded By	CK	CK	CK			
Date 1985	5-31	5-31	5-31			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: slh Date: 6-3-85 Eye Irritation Score: D.D slh

(1311A)

001088

Primary Eye Irritation Test Observations

Test Compound I-3752 RT No. 50503501

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 72 hours

Animal No./ Ear Tag No. FO-	8711	8705	8721			
Location of Corneal Lesions Tail <----------> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NEG	NEG	NEG			
Technician	SAM	SAM	SAM			
Recorded By	SAM	SAM	SAM			
Date 1985	6/1	6/1	6/1			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: SLH Date: 6-3-85 Eye Irritation Score: 0.0 SLH

(1311A)

HAZLETON LABORATORIES AMERICA, INC.
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

PROTOCOL TP2072

Primary Eye Irritation Study in Rabbits (OECD Guidelines)

Study No. 50503501

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

May 21, 1985

PROTOCOL TP2072

Primary Eye Irritation Study in Rabbits
(OECD Guidelines)

Study No.	50503501
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	T-3752
Sponsor's Representative	Janine Gleason
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of May 27, 1985
Completion Date	Week of June 3, 1985
Final Report Date	Week of June 24, 1985

OBJECTIVE

The objective of this study is to determine the level of irritation produced following a single exposure of a test material to one eye of albino rabbits. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/Corrosion, Adopted May 12, 1981[1] and the Principles of Good Laboratory Practice.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Identification

Test Material:	T-3752.
Physical Description:	Brown granular solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be returned to the Sponsor 30 days after issuance of final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

TEST SYSTEM

Test Animal

Young adult albino rabbits of either sex of the New Zealand White strain, approximately 14 weeks of age, will be obtained from Hazleton Research Products, Inc., Denver, Pennsylvania. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Acclimation

Upon receipt, the animals will be taken to a designated animal room where they will be acclimated for at least 1 week before being placed on test (OP-GENB 36). During acclimation, the animals will be examined for clinical abnormalities indicative of health problems (e.g., diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, evidence of injury, etc.). Any animals regarded as unsuitable for the study purposes because of poor physical condition will not be released from acclimation and the reason(s) will be documented.

Identification

Each animal in the study will be assigned a permanent identification number and will be identified with a metal ear tag (OP-GENB 24). All data collected from an animal will be recorded and filed under its identification number.

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 21°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Purina High Fiber Rabbit Chow. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

Study Design

Three rabbits will be selected at random based upon health and a body weight of 2.0 to 3.5 kg.

PROCEDURES

Preparation and Administration of Test Material

The rabbits' eyes will be examined using fluorescein dye procedures within 24 hours prior to test material administration. Only animals with no sign of

corneal injury or eye abnormalities will be utilized. One eye of each animal will be treated with the test material and the other eye will serve as the untreated control.

Each rabbit will receive 0.1 g (or the weight equivalent of 0.1 mL) of solid test material. If necessary, the solid test materials will be finely ground into a dust or powder. The test material will be placed into the everted lower lid of the rabbit's eye. The upper and lower lids are then to be gently held together for 1 second before releasing to prevent loss of material. The eyes of the rabbits will remain unflushed for 24 hours following instillation of the test material. After 24 hours, a washout may be used if considered appropriate.

Reason for Route of Administration

Historically, the route of choice based on the method of Draize.[4]

Observations

The treated eyes of all animals will be examined for ocular irritation at 1, 24, 48, and 72 hours after treatment. If no irritation or injury is present at 72 hours, the study will be terminated. If irritation is present at 72 hours, additional observations will be made at 96 hours and at 7, 14, and 21 days. If at any of these time points there is no irritation, the study will be terminated. If injury is still present at 21 days, the Sponsor will be contacted to determine whether the study should continue or be terminated. After recording the 24-hour observations, sodium fluorescein may be used to aid in revealing possible corneal injury. Irritation will be graded and scored using the Draize technique (Attachment 1).[4] All eye abnormalities will be recorded.

All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.

Body weights will be recorded prior to test material administration and at weekly intervals throughout the study. Observations and body weights will be recorded in the study notebook.

Pathology

All animals, whether dying or sacrificed at study termination, will be discarded.

Report

The final report will present a description of the test material, a description of the test system, dates of study initiation and termination, a summary table showing the irritation data at each observation period, and any special observations that were recorded.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. "Acute Eye Irritation/Corrosion," OECD Guidelines for Testing Chemicals, Section 405 (May 12, 1981).

2. Organisation for Economic Cooperation and Development's Principles of Good Laboratory Practice, Annex 2, 1981.

3. DHEW Publications No. (NIH) 78-23 (1978).

4. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 49-51 (1959).

PROTOCOL APPROVAL

Janine Gleason
Janine Gleason
Sponsor's Representative
3M

5/24/85
Date

Steven M. Glaza
Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

5-22-85
Date

(1109S/tji)

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
2301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

ILLAS D. ZIMMERMAN
NNESOTA MINING & MANUFACTURING COMPANY
IXICOLOGY SERVICES
. PAUL, MN 55101

SAMPLE NUMBER: 50202473
SAMPLE ENTERED: 02/15/85
REPORT PRINTED: 05/07/85

IMPLE: T-3727

IRCHASE ORDER NUMBER: T357842, REL. #505

ENCLOSED: ACUTE ORAL TOXICITY - METHOD, SUMMARY, PATHOLOGY
PRIMARY DERMAL IRRITATION - METHOD, SUMMARY
PRIMARY EYE IRRITATION - METHOD, SUMMARY
QAU REPORT
RAW DATA APPENDIX

SIGNED: Steven M. Glaza
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

MPLE NUMBER: 50202473 PAGE 2

MPLE: T-3727

CD ORAL SCREEN

Objective: To determine the acute oral toxicity produced when a test material is administered by oral gavage to rats according to the Organisation of Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 401, Acute Oral Toxicity, adopted May 12, 1981.

Test Material: T-3727
Physical Description: Off-white waxy solid
Stability of Test Material: Sponsor has purity and stability determinations on file.

Test Animal: Young adult male and female albino rats (approximately 7 weeks of age) of the Sprague-Dawley strain were procured, maintained in group cages in temperature- and humidity-controlled quarters, provided continuous access to commercial laboratory feed and water, and held for an acclimation period of at least 7 days.

Acclimated animals were chosen at random for the study. Test animals were housed by sex in groups of five and identified by animal number and corresponding ear tag. Food and water were available ad libitum throughout the study, except for an overnight period just before test material administration when food, but not water, was withheld.

Reason for Species Selection: The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Method: Five male and five female rats weighing between 200 and 298 g were used for each dosage level. The study consisted of four dosage levels (0.20, 0.50, 2.00 and 5.00 g/kg).

Preparation and Administration of Test Material: For each dose level, the test material was mixed with corn oil and heated in a water bath to form a uniform suspension at a specified concentration. Each suspension was allowed to cool prior to dosing. An individual dose was calculated for each animal based upon its fasted body weight and was administered by gavage. The dose volume of each test mixture was 10.0 ml/kg of body weight.

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

MPLE: T-3727

CD ORAL SCREEN (CONTINUED)

Observations: The animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours following test material administration. The animals were observed daily thereafter for 14 days for clinical signs and twice daily for mortality.

All animals were weighed just before test material administration, at 7 days and at study termination. At the end of the study an acute oral LD50 was calculated for each sex.

Pathology: At study termination surviving animals were euthanatized. Animals which died during the study or were euthanatized received a gross necropsy examination and all abnormalities were recorded.

IMPLE NUMBER: 50202473 PAGE 4

IMPLE: T-3727

:CD ORAL SCREEN (CONTINUED)

SUMMARY

Test Animal: Albino Rats - Sprague-Dawley strain
Source: Harlan Sprague-Dawley, Madison WI
Date Animals Received: 01/22, 02/19 and 03/19/85

Temperature and Humidity of Animal Room: 21 to 25 Degrees C.;
42 to 54% Relative Humidity

Vehicle: Corn oil
Method of Administration: Oral Gavage

Date Test Started: 03/01/85 Date Test Completed: 04/09/85

Estimated Oral LD50*: Male - 0.28 g/kg of body weight
95% Confidence Limits of 0.15 to 0.51 g/kg
Female - 0.43 g/kg of body weight
95% Confidence Limits of 0.19 to 0.97 g/kg

Mortality Summary (Number of Deaths)

Dosage Level (g/kg)	Hours 0 - 4		Days 1		2		3		4		5		6		7-14		Total		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Both
0.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1/5	0/5	1/10
0.50	0	0	0	0	1	0	1	0	1	1	0	0	0	0	2	2	5/5	3/5	8/10
2.00	0	0	2	3	3	2	-	-	-	-	-	-	-	-	-	-	5/5	5/5	10/10
5.00	0	0	3	2	2	3	-	-	-	-	-	-	-	-	-	-	5/5	5/5	10/10

	Dosage Level (g/kg)	Average Body Weights (g) Initial	Day 7	Terminal
Male	0.20	262	277	346
	0.50	271	236	---
	2.00	254	---	---
	5.00	249	---	---
Female	0.20	225	220	240
	0.50	226	189	220
	2.00	242	---	---
	5.00	229	---	---

*Thakur, A. K., and W. L. Fazio, 1981. A computer program for estimating LD50 and its confidence limits using a modified Behrens-Reed-Muench cumulant method. Drug and Chemical Toxicology 4 (3) 297-305.

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CD ORAL SCREEN (CONTINUED)

Clinical Signs

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 0.20 g/kg																	
Males																	
Appeared normal	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	2	0	0	1	1	1	0	0	0	0	0	0	0	0
Dark/red/brown-stained anal/ genital area	0	0	0	4	4	4	4	4	4	3	3	2	2	2	1	1	1
Red-stained face	0	0	0	1	4	3	3	3	3	0	0	1	1	1	0	0	0
Ocular discharge	0	0	0	0	1	2	2	2	2	0	0	0	0	0	0	0	0
Hypoactivity	0	0	0	3	3	3	4	4	4	0	0	0	0	1	0	0	0
Ataxia	0	0	0	0	0	0	2	2	2	0	0	0	0	1	0	0	0
High Carriage	0	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0
Hypersensitivity to touch	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Hyperactivity	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
Clonic convulsions	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Alopecia in abdominal region	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4	4
Red-stained abdomen	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
Piloerection	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0
Yellow-stained genital area	0	0	0	1	1	1	2	2	2	1	1	1	1	1	1	1	1
Swollen genitals	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Prostration	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Death	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0

*Animal died in p.m.

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CD ORAL SCREEN (CONTINUED)

Clinical Signs (continued)

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 0.20 g/kg																	
Females																	
Appeared normal	5	5	5	1	1	0	0	0	0	3	4	4	4	4	4	4	4
Diarrhea	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypoactivity	0	0	0	0	1	2	3	4	4	0	0	0	0	0	0	0	0
Ataxia	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Red-stained face	0	0	0	4	4	3	3	3	3	0	0	0	0	0	0	0	0
Dark-stained anal area	0	0	0	0	2	2	2	2	2	0	0	0	0	0	0	0	0
Yellow-stained abdomen/ genital area	0	0	0	1	1	1	2	2	2	1	0	0	0	0	0	0	0
Red-stained genitals	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
Alopecia in abdominal region	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
Ocular discharge	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0
Piloerection	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0

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CD ORAL SCREEN (CONTINUED)

Clinical Signs (continued)

	Hours			Days													
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 0.5 g/kg							Males										
Appeared normal	4	3	2	0	0	0	0	0	0	0	0	-	-	-	-	-	-
Diarrhea	1	2	3	4	4	3	0	0	0	0	0	-	-	-	-	-	-
Hypoactivity	0	0	0	5	4	3	2	2	2	1	0	-	-	-	-	-	-
Ataxia	0	0	0	1	2	1	0	0	0	0	0	-	-	-	-	-	-
Dyspnea	0	0	0	1	0	0	0	0	0	0	0	-	-	-	-	-	-
Red ocular discharge	0	0	0	0	0	1	0	0	0	0	0	-	-	-	-	-	-
Red-stained face	0	0	0	4	4	3	1	1	1	0	0	-	-	-	-	-	-
Brown-stained anal area	0	0	0	4	4	3	2	2	2	1	0	-	-	-	-	-	-
Red-stained genital region	0	0	0	0	2	2	1	0	0	0	0	-	-	-	-	-	-
Hypersensitivity to touch	0	0	0	0	0	0	0	1	1	0	0	-	-	-	-	-	-
Piloerection	0	0	0	0	0	0	0	0	1	1	0	-	-	-	-	-	-
Thin appearance	0	0	0	0	0	1	0	0	0	0	0	-	-	-	-	-	-
Death	0	0	0	0	1	1	1	0	0	1	1	-	-	-	-	-	-
							Females										
Appeared normal	4	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	1	2	1	4	5	3	0	0	0	0	0	0	0	0	0	0	0
Hypoactivity	0	0	0	5	5	5	4	2	2	2	2	2	2	2	1	2	1
Ataxia	0	0	0	0	0	0	1	2	2	2	2	2	3	3	2	2	1
Convulsions	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Subconvulsive jerking	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hypersensitivity to touch	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	2	1
Piloerection	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	2	1
Brown-stained anal area	0	0	0	3	4	5	4	4	4	4	4	4	4	4	3	2	2
Red-stained genital region	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Red ocular discharge	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0
Red-stained face	0	0	0	3	3	4	2	2	2	2	2	2	3	3	2	2	1
Thin appearance	0	0	0	0	0	0	0	0	0	0	0	1	3	3	2	2	1
Death	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1*	0

*Animal died in p.m.

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ICD ORAL SCREEN (CONTINUED)

Clinical Signs (continued)

	Hours			Days												
	1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dosage Level - 2.00 g/kg																	
Males																	
Appeared normal	3	2	2	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	2	3	3	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Brown-stained anal/ genital area	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	2	3	-	-	-	-	-	-	-	-	-	-	-	-
Females																	
Appeared normal	3	2	2	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	2	3	3	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	0	0	4	0	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	0	0	0	4	0	-	-	-	-	-	-	-	-	-	-	-	-
Red-stained face	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Dyspnea	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Yellow-stained abdomen/anal/ genital area	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	3*	2	-	-	-	-	-	-	-	-	-	-	-	-
Dosage Level - 5.00 g/kg																	
Males																	
Appeared normal	5	4	4	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	1	1	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Brown-stained anal region	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	2	3	-	-	-	-	-	-	-	-	-	-	-	-
Females																	
Appeared normal	4	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	0	4	4	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	1	1	1	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Ataxia	0	0	0	3	0	-	-	-	-	-	-	-	-	-	-	-	-
Dark-stained nose and mouth	0	1	2	1	0	-	-	-	-	-	-	-	-	-	-	-	-
Death	0	0	0	2	3	-	-	-	-	-	-	-	-	-	-	-	-

*Two animals died in p.m.

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CD ORAL SCREEN (CONTINUED)

PATHOLOGY

Dosage Level: 0.20 g/kg of body weight Date Dosed: 03/26/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C28605	M	-	14	Diffuse alopecia on ventral abdominal region.
C28574	M	-	14	No visible lesions.
C28604	M	-	14	No visible lesions.
C28547	M	11	-	Red perinasal discharge; perineum stained brown.
C28414	M	-	14	Diffuse alopecia on ventral abdominal region.
C28329	F	-	14	No visible lesions.
C28394	F	-	14	No visible lesions.
C28395	F	-	14	No visible lesions.
C28346	F	-	14	No visible lesions.
C28399	F	-	14	No visible lesions.

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CD ORAL SCREEN (CONTINUED)

PATHOLOGY (continued)

Dosage Level: 0.50 g/kg of body weight Date Dosed: 03/12/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C29302	M	4	-	Periocular, perinasal, and perineal areas stained dark brown; lungs - diffusely dark red.
C29313	M	2	-	Stomach - multiple, dark brown foci, up to 3 mm in length, on glandular mucosa.
C27575	M	3	-	Dark red periocular discharge (bilateral); perineum - stained dark green; stomach - glandular mucosa diffusely red, with multiple, dark green foci, pinpoint up to 1 mm in diameter, on nonglandular mucosa.
C28418	M	8	-	Stomach - raised, tan areas, up to 2 x 2 x 1 mm, on nonglandular mucosa.
C27576	M	7	-	Perineum/perianal area stained dark brown; stomach - contains dark brown material, glandular mucosa diffusely red, with multiple, raised areas, up to 1 mm in diameter, on nonglandular mucosa; small intestine - contains dark brown, mucoid material.
C28529	F	12	-	Stomach - dark brown areas, up to 1 x 5 mm, on glandular mucosa, with raised, white areas, up to 1 x 3 x 2 mm, on nonglandular mucosa.
C28534	F	-	14	No visible lesions.
C28537	F	4	-	Brown perinasal stain; stomach - dark brown foci, up to 2 mm in diameter, on glandular mucosa.
C28532	F	-	14	No visible lesions.
C28533	F	13	-	Animal thin; stomach - multiple brown areas on glandular mucosa; liver - accentuated lobular pattern on all lobes.

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ICD ORAL SCREEN (CONTINUED)

PATHOLOGY (continued)

Dosage Level: 2.00 g/kg of body weight Date Dosed: 03/06/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C29326	M	1	-	Stomach - contains normal food and tan granular material; small intestine - filled with tan/yellow, mucoid semifluid.
C29328	M	1	-	Stomach - contains normal food and tan granular material; small intestine - filled with tan/yellow, mucoid semifluid.
C29330	M	2	-	Stomach - glandular mucosa diffusely red; liver - accentuated lobular pattern.
C29335	M	2	-	Liver - accentuated lobular pattern.
C29331	M	2	-	No visible lesions.
C28664	F	1	-	Stomach - contains normal food and tan granular material; small intestine - filled with tan and clear, mucoid semifluid.
C28497	F	1	-	No visible lesions.
C28695	F	2	-	No visible lesions.
C28693	F	2	-	Liver - accentuated lobular pattern.
C28687	F	1	-	Liver - accentuated lobular pattern.

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CD ORAL SCREEN (CONTINUED)

PATHOLOGY

Dosage Level: 5.00 g/kg of body weight Date Dosed: 03/01/85

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Comments
C28545	M	1	-	Perineum - stained brown.
C29314	M	1	-	Perineum - stained brown.
C28198	M	1	-	Perineum - stained brown.
C29319	M	2	-	Red perinasal discharge.
C28456	M	2	-	Perineum - stained brown; red periocular discharge (bilateral); red perinasal discharge.
C28498	F	1	-	Perineum - stained brown; lungs - dark red and firm; thoracic cavity - contains a light tan granular material.
C28501	F	1	-	Perineum - stained brown.
C28500	F	2	-	Perineum - stained brown; red perinasal discharge.
C28499	F	2	-	Perineum - stained brown; red perinasal discharge.
C28503	F	2	-	Perineum - stained brown; red perinasal discharge.

Deviations from the protocol: Some rats received a commercial laboratory feed other than Purina Rodent Chow. During the study period the temperature of the animal room ranged from 21 to 25 degrees C. These deviations are not considered to have had an effect on the validity of the study.

References: Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, Acute Oral Toxicity, adopted May 12, 1981.

CD SKIN IRRITATION

Objective: To determine the relative level of primary skin irritation/corrosion of a test substance on rabbits under semioccluded conditions according to the Organisation of Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 404, Acute Dermal Irritation/Corrosion, adopted May 12, 1981.

Test Material: T-3727
Physical Description: Off-white waxy solid
Purity and Stability: Sponsor has purity and stability determinations on file.

Test Animal: Young adult rabbits (approximately 14 weeks of age) of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided continuous access to Teklad Laboratory Rabbit Diet and water, and held for an acclimation period of at least 7 days.

Three acclimated female animals, weighing from 2840 to 3112 g, were chosen at random for the test, treated, and maintained during the observation period as specified for the acclimation period. Test animals were identified by animal number and corresponding ear tag. Approximately twenty-four hours before treatment the hair was clipped from the back of each animal.

Reason for Species Selection: Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

Preparation of Test Material: The sample was dosed as received.

Treatment: The test material was applied to the intact skin of each rabbit in the amount of 0.5 g per site and moistened with 0.9% saline. The treated area was covered with a 2.5 x 2.5-cm gauze patch secured with paper tape and overwrapped with Saran Wrap and Elastoplast tape to provide a semiocclusive dressing. Collars were used to restrain the test animals for the 4-hour exposure period.

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CD SKIN IRRITATION (CONTINUED)

Observations: After the exposure period, the patches were removed. The test sites were washed using lukewarm tap water and disposable paper towels. The test material was removed from the test sites as thoroughly as possible without irritating the skin. Thirty minutes following removal of the test material, the degree of erythema and edema was read according to the Draize* technique. Subsequent examinations were made at 24, 48 and 72 hours after patch removal.

Individual body weights were taken just prior to study initiation.

Pathology: At study termination all animals were euthanatized and discarded.

*Draize, J. H., "Appraisal of The Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

MPLE NUMBER: 50202473

MPLE: T-3727

CD SKIN IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino Rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 02/05/85

Temperature and Humidity of Animal Room: 20 - 22 Degrees C.;
40 - 44% Relative Humidity

Date Test Started: 03/01/85 Date Test Completed: 03/04/85

Individual Dermal Irritation Scores
Test Material: T-3727

Animal Number	Erythema Score Hours 4	24	48	72	Edema Score Hours 4	24	48	72
F07819	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F07816	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F07800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Primary Dermal Irritation Scores

Observation Period	3 Rabbit Mean
4 Hours:	0.0
24 Hours:	0.0
48 Hours:	0.0
72 Hours:	0.0

Results:

No dermal irritation was observed at any time during the study period.

Deviation from the protocol: The test material was moistened with 0.9% saline rather than deionized water as stated in the protocol. This deviation is not considered to have had an effect on the validity of the study.

CD SKIN IRRITATION (CONTINUED)

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 404, Acute Dermal Irritation/ Corrosion, adopted May 12, 1981.

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

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AMPLE: T-3727

ICD EYE IRRITATION

Objective: To determine the level of ocular irritation produced following a single exposure of a test substance to one eye of albino rabbits according to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/ Corrosion, adopted May 12, 1981.

Test Material: T-3727
Physical Description: Off-white waxy solid
Purity and Stability: Sponsor has purity and stability determinations on file.

Test animal: Young adult rabbits (approximately 14 weeks of age) of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided continuous access to Teklad Laboratory Rabbit Diet and water, and held for an acclimation period of at least 7 days.

Three acclimated female animals, weighing from 2666 to 3000 g, were chosen at random for the test. The animals' eyes were examined within 24 hours prior to test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used. Test animals were identified by animal number and corresponding ear tag.

Reason for Species Selection: The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Preparation of Test Material: The sample was dosed as received. A bulk density determination was made to determine the weight equivalent of a 0.1 ml dose. Based upon the density determination, an individual dose of 0.09 g was weighed out for each animal.

Treatment: Each rabbit received 0.09 g (0.1 ml weight equivalent) of the test material placed on the everted lower lid of one eye, with the contralateral eye serving as the untreated control. The upper and lower lids were gently held together for one second to prevent loss of material and then released. The eyes of the rabbits remained unflushed.

MPLE: T-3727

CD EYE IRRITATION (CONTINUED)

Observations: The treated eyes were observed for ocular irritation at 1, 24, 48, 72 and 96 hours after treatment. At the 72-hour reading, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize* technique.

Animals were weighed just prior to test material administration.

Pathology: At study termination all animals were euthanatized and discarded.

*Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 49-51 (1959).

AMPLE NUMBER: 50202473

AMPLE: T-3727

CD EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 02/05/85

Temperature and Humidity of Animal Room: 19 to 22 Degrees C.;
40 to 44% Relative Humidity

Test Material: T-3727

Date Test Started: 02/28/85 Date Test Completed: 03/04/85

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	3 Rabbit Mean 0.09 g (Unwashed)
1 Hour:	7.0
24 Hours:	3.7
48 Hours:	3.0
72 Hours:	2.3
96 Hours:	0.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (3) at each observation period.

Comments: No pain response (vocalization) was elicited from any animal following instillation of the test material.

No corneal irritation was observed during the study.

MPLE NUMBER: 50202473

MPLE: T-3727

CD EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F07813	1 Hour	0	0	0	1	5	1	1	1	6
	24 Hours	0	0	0	1	5	1	1	0	4
	48 Hours	0	0	0	1	5	1	1	0	4
	72 Hours	0	0	0	1	5	1	0	0	2
	96 Hours	0	0	0	0	0	0	0	0	0
F07814	1 Hour	0	0	0	0	0	1	1	1	6
	24 Hours	0	0	0	0	0	1	0	0	2
	48 Hours	0	0	0	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0	0	0	0
	96 Hours	0	0	0	0	0	0	0	0	0
F07815	1 Hour	0	0	0	0	0	1	1	0	4
	24 Hours	0	0	0	0	0	0	0	0	0
	48 Hours	0	0	0	0	0	0	0	0	0
	72 Hours	0	0	0	0	0	0	0	0	0
	96 Hours	0	0	0	0	0	0	0	0	0

Table 2
Sodium Fluorescein Examination

Animal Number	Observation Period Pre-initiation	Observation Period 72 Hours
F07813	NEG	NEG
F07814	NEG	NEG
F07815	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/Corrosion, adopted May 12, 1981.

2. Draize J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity", Association of Food and Drug Official Officials of the United States, Topeka, Kansas, pp. 49-51 (1959).

QUALITY ASSURANCE STATEMENT

Study No. 50202473

The report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 21 CFR 58.35 (b) (6) (7). It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study and that the reported data accurately reflect the raw data of the laboratory study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Date of Inspection	Type of Inspection	Date Issued to Management
Acute Oral Toxicity Study in Rats		
2/25-26/85	Process Audit	2/26/85
3/26/85	Process Audit	3/26/85
5/01/85	Report Review	5/01/85
Primary Dermal Irritation Study in Rabbits		
2/25-26/85	Process Audit	2/26/85
5/01/85	Report Review	5/01/85
Primary Eye Irritation Study in Rabbits		
2/25-26/85	Process Audit	2/26/85
5/01/85	Report Review	5/01/85

Diana E. Skalitzky
Inspector, Quality Assurance Unit

5/2/85
Date

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3727 Vehicle CORN OIL RT No. 50202473

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 3-19-85

Fasted: Date 3-25-85 Time 2:30 p.m. Tech. CK Room No. 3

♂

Sex ♂									Tech.	1985 Date	Ktron Scale Used:
Dosage 0.20 (g/kg)											
Dose Volume 10.0 (ml/kg)	Dose Time 11:55 a.m.										
Animal No./Ear Tag No. C2	8605	8574	8604	8547	8414	8576	7792		Sam	3-26	NA
Prefasted Body Weight (g)	NA										
Fasted Body Weight (g)	244	250	278	279	260	258	247		Sam	3-26	KTRON 5228
Actual Dose (ml)	2.4	2.5	2.8	2.8	2.6	2.6	2.5		Sam	3-26	NA
Day 7 Body Weight (g)	254	297	324	258	252	*	*		① Sam / jp	3-26 / 4-2	15019
Day 14 Body Weight (g)	327	362	379	DEAD 4-6-85	317	*	*		jp / SP	4-9	Ktron 15019
				SP 210g		Doses Verified by			pgr	4/9	NA

① Recording error 3-26-85 Sam

♀

Dosage 0.20 (g/kg)											Ktron
Dose Volume 10.0 (ml/kg)	Dose Time 12:00 p.m.								Tech.	1985 Date	Scale Used:
Animal No./Ear Tag No. C2	8329	8394	8402	8395	8346	8381	8399		Sam	3-26	NA
Prefasted Body Weight (g)	NA										
Fasted Body Weight (g)	234	218	211	200	229	215	242		Sam	3-26	KTRON 5228
Actual Dose (ml)	2.3	2.2	2.1	2.0	2.3	2.2	2.4		Sam	3-26	NA
Day 7 Body Weight (g)	284	204	*	190	204	*	249		jp	4-2	15019
Day 14 Body Weight (g)	270	218	*	209	241	*	261		jp / SP	4-9	15019
						Doses Verified by			pgr	4/9	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	Study Day 1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6 am	6 pm	7 am	7 pm	8 am	8 pm	9 am	9 pm	10 am	10 pm	11 am	11 pm	12 am	12 pm	13 am	13 pm	14 am	14 pm	Total
♂ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	1/5
♀ 0.20g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA	0/5
Technician	Sam	Sam	Sam	CK	CK	CK	CK	Sam	Sam	Sam	Sam	CK	CK	jp	jp	SP	SP	SP	SP	CK	CK	SP	SP	SP	SP	CK	CK	SP	NA	pgr
Date	3/26	3/27	3/27	3/26	3/26	3/29	3/29	3/30	3/30	3/31	3/31	4/1	4/1	4/2	4/2	4/3	4/3	4/4	4/4	4/6	4/5	4/6	4/6	4/7	4/7	4/8	4/8	4/9	NA	4/9

NA - Not Applicable

* - Dosage calculated, but not administered
Unused animal returned to stock

Reviewed by pgr Date 4/9/85

C01121

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3727 Vehicle CORN OIL RT No. 5D202473

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 1-22-85

Fasted: Date 3-11-85 Time 3:00 p.m. Tech. SRM Room No. 3

Sex ♂

Dosage 0.50 (g/kg)								Tech.	1985 Date	Scale Used:
Dose Volume 10.0 (ml/kg)	Dose Time 9:25 a.m.									
Animal No./Ear Tag No. C2	9302	9313	7575	9318	7576	8418		SRM	3-12	NA
Prefasted Body Weight (g)	NA									→
Fasted Body Weight (g)	255	274	256	226	274	298		SRM	3-12	KTRON 5228
Actual Dose (ml)	2.6	2.7	2.6	2.3	2.7	① 3.0		SRM	3-12	NA
Day 7 Body Weight (g)	Dead 3-16-85	DEAD 3-14-85 SRM	DEAD 3-15-85 SP	*	Dead 3-19-85 CK	236		CK	3-19	Ktron 5228
Day 14 Body Weight (g)	185g NP	235g SRM	216g SP	*	206g CK	DEAD 3-30-85 SRM				
						Dosed Verified by Body wt. 227g		MP	3-12	NA

① writing errors 3-12-85 SRM

Sex ♀

Dosage 0.50 (g/kg)								Tech.	1985 Date	Scale Used:
Dose Volume 10.0 (ml/kg)	Dose Time 9:30 a.m.									
Animal No./Ear Tag No. C2	8529	8534	8537	8532	8531	8533		SRM	3-12	NA
Prefasted Body Weight (g)	NA									→
Fasted Body Weight (g)	231	236	223	232	211	206		SRM	3-12	KTRON 5228
Actual Dose (ml)	2.3	2.4	2.2	2.3	2.1	2.1		SRM	3-12	NA
Day 7 Body Weight (g)	160	209	Dead 3-16-85	193	*	192		CK	3-19	Ktron 5228
Day 14 Body Weight (g)	Found Dead 3-24-85 CK 160g	223	175g NP	217	*	DEAD 3-25-85 SP 184g		CK	3-26	Ktron 5228
						Dosed Verified by		MP	3-12	NA

① writing error 3-14-85 SRM

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours	Study Day																														Total
	0 - 4	1		① 2		3		4		5		6		7		8		9		10		11		12		13		14				
		am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm		
♂ 0.50g/kg	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	NA	→										→	NA	5/5	
♀ 0.50g/kg	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	2/5	1/5	0/5	NA	3/5	
Technician	SRM	SP	SP	SRM	SRM	SP	SP	MP	MP	MP	MP	CK	CK	CK	CK	SRM	SRM	CK	CK	SP	CK	CK	CK	CK	CK	SP	SP	CK	NA		pgw
Date 1985	3/12	3/13	3/13	3/14	3/14	3/15	3/15	3/16	3/16	3/17	3/17	3/19	3/16	3/19	3/19	3/20	3/20	3/21	3/21	3/22	3/22	3/23	3/23	3/24	3/24	3/25	3/25	3/26	NA	4/10	

NA - Not Applicable

* - Dosage calculated, but not administered
Unused animal returned to stock

Reviewed by pgw Date 4/10/85

GU122

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3727 Vehicle CORN OIL RT No. 50202473

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 1-22-85 & 2-19-85

Fasted: Date 3-5-85 Time 2:00 pm Tech. CK Room No. 3

♂

Dosage 2.00 (g/kg)										
Dose Volume 10.0 (ml/kg)	Dose Time 10:45 AM							Tech.	Date 1985	Scale Used:
Animal No./Ear Tag No. C2	9326	9330	9329	9335	9328	9327	9331	SMM	3-6	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	267	268	272	270	219	247	248	SMM	3-6	KTRON 1348
Actual Dose (ml)	2.7	2.7	2.7	2.7	2.2	2.5	2.5	SMM	3-6	NA
Day 7 Body Weight (g)	Dead 249g	DEAD 3-8-85 SP	misdosed 3-6-85 SMM	DEAD 3-8-85 SP	Dead 207	misdosed 3-6-85 SMM	DEAD 3-8-85 SP			
Day 14 Body Weight (g)	3-7-85 CBS KTron 1348	243 g		246 g	3-7-85 CBS KTron 1348		221 g			
							Doses Verified by	MP	3-6	NA

♀

Dosage 2.00 (g/kg)										
Dose Volume 10.0 (ml/kg)	Dose Time 11:00 AM							Tech.	Date 1985	Scale Used:
Animal No./Ear Tag No. C2	8663	8664	8497	8695	8692	8693	8687	SMM	3-6	NA
Prefasted Body Weight (g)	NA									
Fasted Body Weight (g)	228	251	233	253	209	247	224	SMM	3-6	KTRON 1348
Actual Dose (ml)	2.3	2.5	2.3	2.5	2.1	2.5	2.2	SMM	3-6	NA
Day 7 Body Weight (g)	*	Dead 235g	Dead 211g	DEAD 3-8-85	*	DEAD 3-8-85	Dead 211g			
Day 14 Body Weight (g)	*	3-7-85 CBS KTron 1348	3-7-85 CBS KTron 6238	SP 230g	*	SP 223g	3-7-85 CBS KTron 6238			
							Doses Verified by	MP	3-6	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	Study Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
		am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	am / pm	
♂ 2.00 g/kg	0/5	0/5 / 0/5	NA ②												→ NA	5/5
♀ 2.00 g/kg	0/5	0/5 / 0/5	NA ②												→ NA	5/5
Technician	SMM	SMM / SMM	NA												→ NA	SLH
Date 1985	3/6	3/7 / 3/7	NA												→ NA	3/11

NA - Not Applicable

* - Dosage calculated, but not administered
Unused animal returned to stock

① Entry errors 3-7-85 SMM

Reviewed by SLH Date 3-11-85

② Inadvertenly not recored mortality for study day 2 SP 3-11-85

C01123

ACUTE ORAL TOXICITY (LD_{50}) RECORD

Test Material T-3727 Vehicle Corn oil RT No. 50202473

Bulk Density NA (g/ml) Species Rat Source Harlan Date Received 1-22-85

Fasted: Date 2-28-85 Time 5:00 pm Tech. SRM Room No. 3

Sex	Dosage 5.00 (g/kg) / Dose Volume 10.0 (ml/kg)								Dose Time 11:45 AM	Tech.	1985 Date	Scale Used:
♂	Animal No./Ear Tag No. C2	8545	8456	9314	NA	8198	9313	9319		SRM	3/1	NA
	Prefasted Body Weight (g)	NA										
	Fasted Body Weight (g)	287	264	236	266①	247	235	210		SRM	3/1	KIRON 15019
	Actual Dose (ml)	2.9	2.6	2.4	2.7	2.5	2.4	2.1		SRM	3/1	NA
	Day 7 Body Weight (g)	Dead	Dead	Dead	NA	Dead	*	Dead				
	Day 14 Body Weight (g)	Wt 278g 3-3-85 MP	Wt 244g 3-3-85 MP	Wt 227g 3-2-85 MP	↓	Wt 200g 3-3-85 MP	*	3-3-85 MP 179g		Doses Verified by	3/1	NA

① Animal not used, missing ear tag 3-1-85 SRM

③ Illegible entry, date should be 3/2/85 but not corrected or footnoted until 5-3-85 MP

Sex	Dosage 5.00 (g/kg) / Dose Volume 10.0 (ml/kg)								Dose Time 12:00 AM	Tech.	1985 Date	Scale Used:
♀	Animal No./Ear Tag No. C2	8500	8498	8497	8501	8503	8502	8499		SRM	3/1	NA
	Prefasted Body Weight (g)	NA										
	Fasted Body Weight (g)	237	231	235	230	207	223	242		SRM	3/1	KIRON 15019
	Actual Dose (ml)	2.4	2.3	2.4	2.3	2.1	2.2	2.4		SRM	3/1	NA
	Day 7 Body Weight (g)	Dead	Dead	*	Dead	Dead	*	Dead				
	Day 14 Body Weight (g)	3-3-85 MP 220g	Wt 226g 3-2-85 MP	*	Wt 217g 3-2-85 MP	3-3-85 MP 199g	*	3-3-85 MP 219g		Doses Verified by	3/1	NA

MORTALITY (NO. DIED/NO. DOSED)

Dose Level	Hours 0 - 4	Study Day 1 am	1 pm	2 am	2 pm	3 am	3 pm	4 am	4 pm	5 am	5 pm	6 am	6 pm	7 am	7 pm	8 am	8 pm	9 am	9 pm	10 am	10 pm	11 am	11 pm	12 am	12 pm	13 am	13 pm	14 am	14 pm	Total
♂ 5.00g/kg	0/5	0/5	0/5	0/5	NA																							>	NA	0/5
♀ 5.00g/kg	0/5	0/5	0/5	0/5	NA																							>	NA	0/5
Technician	SRM	MP	MP	MP	NA																							>	NA	SLH
Date 1985	3/1	3/2	3/2	3/3	NA																							>	NA	3/11

NA - Not Applicable

* - Dosage calculated, but not administered
Unused animal returned to stock

Reviewed by SLH Date 3-11-85

C01124

STUDY TITLE: Acute Oral Toxicity

RT NO. 50202473

TEST MATERIAL. T-3727

SEX ♂

DOSAGE LEVEL. 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8547

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6			
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE			
Diarrhea				✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE			
Brown stained anal area				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Red stained face					✓	NE	NE	NE	NE	NE	NE	✓	✓	✓			
ocular discharge					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE			
hypoactive							✓	✓	✓	NE	NE	NE	NE	✓			
High carriage										✓	✓	NE	NE	✓			
Hyperactive										✓	✓	✓	NE	NE			
Hypersensitive to touch											✓	✓	NE	NE			
clonic Convulsion													✓	NE			
Prostration													✓	NE			
Ataxic														✓			
DEATH														①✓			
TECHNICIAN	SMM	SMM	SMM	SMM	CK	CK	SMM	SMM	CK	MO	MO	MO	CK	SP			
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6			

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① DIED IN PM SP 4-6-85

C01125

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8604

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red Stained Face				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
diarrhea				✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Brown Stained anal/genital area				✓	✓	✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE
Alopecia Abdominal region										✓	✓	✓	✓	✓	✓	✓	✓
DEATH													②				
TECHNICIAN	Smm	Smm	Smm	Smm	CK	CK	Smm	Smm	CK	SP	CK	CK	CK SP	SP	SP	SP	SP
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① Entry Errors SP 4-6-85

② observation done on 4/5 not recorded till 4-10-85 CK

C01126

STUDY TITLE: Acute Oral Toxicity

RT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♂

DOSAGE LEVEL: 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8605

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Red stained abdomen				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Red stained back					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Pilo erection						✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained genital area							✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ataxic							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Alopecia abdominal region										~~NE~~ ✓	✓	✓	✓	✓	✓	✓	✓
High carriage										✓	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	CK	CK	[illegible]	[illegible]	CK	[illegible]	[illegible]	[illegible]	CK	SP	SP	SP	[illegible]
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

Dentry error 4-2-85 jp

✓ - Sign Present
sl - Sign Present, Slight
NE - Not Evident
NA - Not Applicable

C01127

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8414

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Red stained genital area				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Dark stained anal area				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red stained face					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
ocular discharge						✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
swollen genitals							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Ataxic							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Alopecia abdominal region										✓	✓	✓	✓	✓	✓	✓	✓
High carriage										✓	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	CK	CK	[illegible]	[illegible]	CK	[illegible]	[illegible]	[illegible]	CK	SP	SP	[illegible]	[illegible]
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

001128

STUDY TITLE: Acute Oral Toxicity

RT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♂

DOSAGE LEVEL: 0.20 g/kg

ANIMAL/KAR TAG NO. C2-8574

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypoactive				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Dark stained anal area				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained genitals				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Alopecia abdominal region										✓	✓	✓	✓	✓	✓	✓	✓
DEATH																	
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	CK	CK	[illegible]	[illegible]	CK	[illegible]	[illegible]	[illegible]	CK	SP	SP	[illegible]	[illegible]
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01429

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8395

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/24	3/24	3/24	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea				✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Hypo active					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
dark stained Anal area					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
piloerection							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Ataxic								✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sann	Sann	Sann	Sann	CK	CK	Sann	Sann	CK	[illegible]	[illegible]	[illegible]	CK	SP	SP	SP	[illegible]
DATE 1985	3/24	3/24	3/24	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01130

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.20g/kg

ANIMAL/EAR TAG NO. C2-8394

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓
Diarrhea				✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face				✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Dark stained anal Area					✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sma	Sma	Sma	Sma	CK	CK	Sma	Sma	CK	JD	DP	DP	CK	SP	SP	JD	JD
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01131

STUDY TITLE: Acute Oral Toxicity

RT NO. 50202473

TEST MATERIAL. T-3727

SEX ♀

DOSAGE LEVEL. 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8399

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/24	3/24	3/24	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red stained face				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Red stained genitals				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained abdomen							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
hypoactive							✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Alopecia abdominal region										✓	✓	✓	✓	✓	✓	✓	✓
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	CK	CK	Sam	Sam	CK	KM	KM	KM	CK	SP	SP	SP	SP
DATE 1985	3/24	3/24	3/24	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01132

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8329

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓
Red stained face				✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
yellow stained genital area				✓	✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE
hypoactive								✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sam	Sam	Sam	Sam	CK	CK	Sam	Sam	CK	MP	MP	MP	CK	SP	SP	SOJ	MP
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01133

STUDY TITLE: Acute Oral Toxicity

BT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.20 g/kg

ANIMAL/EAR TAG NO. C2-8346

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9
APPEARED NORMAL	✓	✓	✓	✓	✓	NE	NE	NE	NE	✓	✓	✓	✓	✓	✓	✓	✓
Hypoactive						✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
Ocular discharge						✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE
DEATH																	
TECHNICIAN	Sman	Sman	Sman	Sman	CK	CK	Sman	Sman	CK	JO	MP	MP	CK	SP	SP	JOS	JO
DATE 1985	3/26	3/26	3/26	3/27	3/28	3/29	3/30	3/31	4/1	4/2	4/3	4/4	4/5	4/6	4/7	4/8	4/9

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01134

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♂

DOSAGE LEVEL: 0.5Dg/Kg

ANIMAL/EAR TAG NO.: C2-8418

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20						
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE						
Diarrhea				✓	✓	✓	NE	NE	NE	NE	NA						
Brown stained Anal Area				✓	✓	✓	✓	✓	✓	✓	NA						
Red Stained Face				✓	✓	✓	NE	NE	NE	NE	NA						
Hypoactive				✓	✓	✓	✓	✓	✓	✓	NA						
Piloerection									✓	✓	NA						
DEATH											✓						
TECHNICIAN	Sm	Sm	Sm	SP	Sm	SP	KP	KP	CK	CK	Sm						
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20						

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01135

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 0.5Dg/Kg

ANIMAL/EAR TAG NO. C2-933

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14												
APPEARED NORMAL	✓	NE	NE	NE	NE												
Diarrhea	—	✓	✓	✓	NA												
Brown Stained Anal Area	—	—	—	✓	NA												
Red stained Face	—	—	—	✓	NA												
Hypoactive	—	—	—	✓	NA												
Ataxic	—	—	—	✓	NA												
Dyspnea	—	—	—	✓	NA												
DEATH					✓												
TECHNICIAN	[illegible]	[illegible]	[illegible]	SP	[illegible]												
DATE 1985	3/12	3/12	3/12	3/13	3/14												

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01136

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♂

DOSAGE LEVEL: 0.5g/kg

ANIMAL/EAR TAG NO. C2-7576

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19							
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE							
Hypoactive				✓	✓	✓	✓	✓	✓	NA							
Red stained face					✓	✓	✓	✓	✓	NA							
Brown stained anal region					✓	✓	✓	✓	✓	NA							
Red stained genital region					✓	✓	✓	NE	NE	NA							
Diarrhea					✓	✓	NE	NE	NE	NA							
Hypersensitive to touch							---	✓	✓	NA							
DEATH										✓							
TECHNICIAN	Gram	Gram	Gram	SP	Gram	SP	DP	DP	CK	CK							
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19							

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01137

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 0.50g/kg

ANIMAL/EAR TAG NO. C2-7575

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15											
APPEARED NORMAL	✓	✓	NE	NE	NE	NE											
Diarrhea	—	—	✓	✓	✓	NA											
Brown stained anal area	—	—	—	✓	✓	NA											
Red Stained Face	—	—	—	✓	✓	NA											
Hypoactive	—	—	—	✓	✓	NA											
Ataxic	—	—	—	—	✓	NA											
DEATH						✓											
TECHNICIAN	Sam	Sam	Sam	SP	Sam	SP											
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15											

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 0.50g/kg

ANIMAL/EAR TAG NO. C2-9302

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16										
APPEARED NORMAL	NO	NE	NO	NE	NE	NE	NA										
Diarrhea	✓	✓	✓	✓	✓	✓	NA										
Brown stained anal area				✓	✓	✓	NA										
Red stained Face				✓	✓	✓	NA										
Hypoactive				✓	✓	✓	NA										
Red stained genital region					✓	✓	NA										
Ataxic					✓	✓	NA										
Appears thin						✓	NA										
Red Ocular discharge						✓	NA										
DEATH							✓										
TECHNICIAN	Sam	Sam	Sam	SP	Sam	SP	NO										
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16										

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01139

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♀

DOSAGE LEVEL: 0.5Dg/Kg

ANIMAL/EAR TAG NO. C2-8534

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/A	3/20	3/21	3/22	3/23	3/24	3/25	3/26
APPEARED NORMAL	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Brown stained Anal Area				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red stained face				✓	✓	✓	NE	NE	NE	NE	NE	NE	✓	✓	✓	✓	✓
Hypoactive				✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Hypersensitive to touch													✓	✓	✓	✓	✓
DEATH																	
TECHNICIAN	SMM	SMM	SMM	SP	SMM	SP	MP	MP	CK	CK	SMM	CK	SP	CK	CK	SP	CK
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24	3/25	3/26

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01140

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.5Dg/Kg

ANIMAL/EAR TAG NO. C2-8529

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24		
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Diarrhea				✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NA		
Brown stained Anal Area				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NA		
Red stained Face				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NA		
Hypoactive				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NA		
Ataxic							✓	✓	✓	✓	✓	✓	✓	✓	NA		
Convulsions											✓	✓	NE	NE	NA		
Hypersensitive to touch													✓	✓	NA		
Piloerection													✓	✓	NA		
Appears thin													✓	✓	NA		
DEATH															✓		
TECHNICIAN	Sam	Sam	Sam	SP	Sam	SP	CK	CK	CK	CK	Sam	CK	SP	CK	CK		
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24		

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01151

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.50g/Kg

ANIMAL/EAR TAG NO. C2-8533

STUDY DAY	HOURS 1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24	3/25	
APPEARED NORMAL	✓	NE	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	
Diarrhea		✓	NE	NE	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	
Red Stained Face				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Hypoactive				✓	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	✓	
Brown stained anal region					✓	✓	✓	✓	✓	✓	✓	✓	✓	NE	✓	NE	
appears thin												✓	✓	✓	✓	✓	
Piloerection													✓	✓	✓	✓	
Ataxic													✓	✓	✓	✓	
Hypersensitive to touch																✓	
DEATH																✓①	
TECHNICIAN	Sm	Sm	Sm	SP	Sm	SP	CK	CK	CK	CK	Sm	CK	SP	CK	CK	SP	
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24	3/25	

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① entry error CK 3-23-85

② DIED IN PM SP 3-25-85

001422

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.50g/kg

ANIMAL/EAR TAG NO. C2-8532

	HOURS																
STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24	3/25	3/26
APPEARED NORMAL	✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Diarrhea				✓	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Brown stained Anal Area				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hypoactive				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red stained genital region				—	✓	✓	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Red Ocular discharge					✓	✓	✓	✓	✓	✓	✓	✓	NE	NE	NE	NE	NE
Ataxic								✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subconvulsive Jerking								✓	NE	NE	NE	NE	NE	NE	NE	NE	NE
Appears thin													✓	✓	✓	✓	✓
Piloerection																✓	✓
DEATH																	
TECHNICIAN	Sam	Sam	Sam	SP	Sam	SP	MD	MD	CK	CK	Sam	CK	SP	CK	CK	SP	CK
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16	3/17	3/18	3/19	3/20	3/21	3/22	3/23	3/24	3/25	3/26

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① Entry Error SP 3-15-85

C011433

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 0.5g/kg

ANIMAL/EAR TAG NO. C2-8537

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/12	3/12	3/12	3/13	3/14	3/15	3/16										
APPEARED NORMAL	✓	✓	✓	NONE		NE	NA										
Diarrhea				✓	✓	✓	NA										
Hyperactive				✓	✓	✓	NA										
Red Stained Face					✓	✓	NA										
Brown Stained anal area						✓	NA										
DEATH							✓										
TECHNICIAN	[illegible]	[illegible]	[illegible]	SP	[illegible]	SP	MP										
DATE 1985	3/12	3/12	3/12	3/13	3/14	3/15	3/16										

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

① Entry Error SP 3-15-85

C011144

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 2.00 g/kg

ANIMAL/EAR TAG NO. C2 9331

	HOURS																	
STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SCHEDULED DATE 1985	3/6	3/6	3/6	3/7														
APPEARED NORMAL	✓	NE	NE	NE	NA													
Diarrhea		✓	✓	NE														
Hypoactive				✓														
Ataxic				✓														
Brown stained abdomen & genital area				✓														
DEATH					✓													
TECHNICIAN	Sam	Sam	Sam	JDS	SP													
DATE 1985	3/6	3/6	3/6	3/7	3/8													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01145

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL. T-3727

SEX ♂

DOSAGE LEVEL. 2.0g/kg

ANIMAL/EAR TAG NO. C29330

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE 1985	3/6	3/6	3/6	3/7	3/8												
APPEARED NORMAL	NE	NE	NE	NE	NA												
Diarrhea	✓	✓	✓	NE													
Hypoactive				✓													
Ataxic				✓													
Brown stained anal area				✓	✓												
DEATH					✓												
TECHNICIAN	[illegible]	[illegible]	[illegible]	MS	SP												
DATE 1985	3/6	3/6	3/6	3/7	3/8												

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 2.0g/kg

ANIMAL/EAR TAG NO. C29326

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE ABS	3/6	3/6	3/6	3/7													
APPEARED NORMAL	✓	✓	✓	NE													
DEATH				✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	3/6	3/6	3/6	3/7													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

001447

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 2.0g/kg

ANIMAL/EAR TAG NO. C29335

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/6	3/6	3/6	3/7	3/8												
APPEARED NORMAL	NE	NE	NE	NE	NA												
Diarrhea	✓	✓	✓	✓	↓												
Hypoactive				✓	✓												
DEATH					✓												
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]	SP												
DATE 1985	3/6	3/6	3/6	3/7	3/8												

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01448

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 2.0g/kg

ANIMAL/EAR TAG NO. C29328

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE PBS	3/6	3/6	3/6	3/7													
APPEARED NORMAL	✓	✓	✓	NE													
DEATH				✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	3/6	3/6	3/10	3/7													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 2.0g/kg

ANIMAL/EAR TAG NO. C29687

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE 1985	3/6	3/6	3/6	3/7													
APPEARED NORMAL	NE	NE	NE	NE													
Diarrhea	✓	✓	✓	✓													
Yellow stained abdomen/genital area				✓													
Hypoactive				✓													
Ataxic				✓													
Lacrimation				✓													
DEATH				✓①													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	3/6	3/6	3/6	3/7													

✓ - Sign Present
sl - Sign Present, Slight
NE - Not Evident
NA - Not Applicable

①Animal died in p.m. 3-7-85 DDS

001150

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473 SEX ♀

TEST MATERIAL. T-3227 DOSAGE LEVEL. 2.0g/kg ANIMAL/EAR TAG NO. C28664...

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE 1985	3/6	3/6	3/6	3/7													
APPEARED NORMAL	✓	✓	✓	NE													
DEATH				✓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1985	3/6	3/6	3/6	3/7													

✓ - Sign Present
sl - Sign Present, Slight

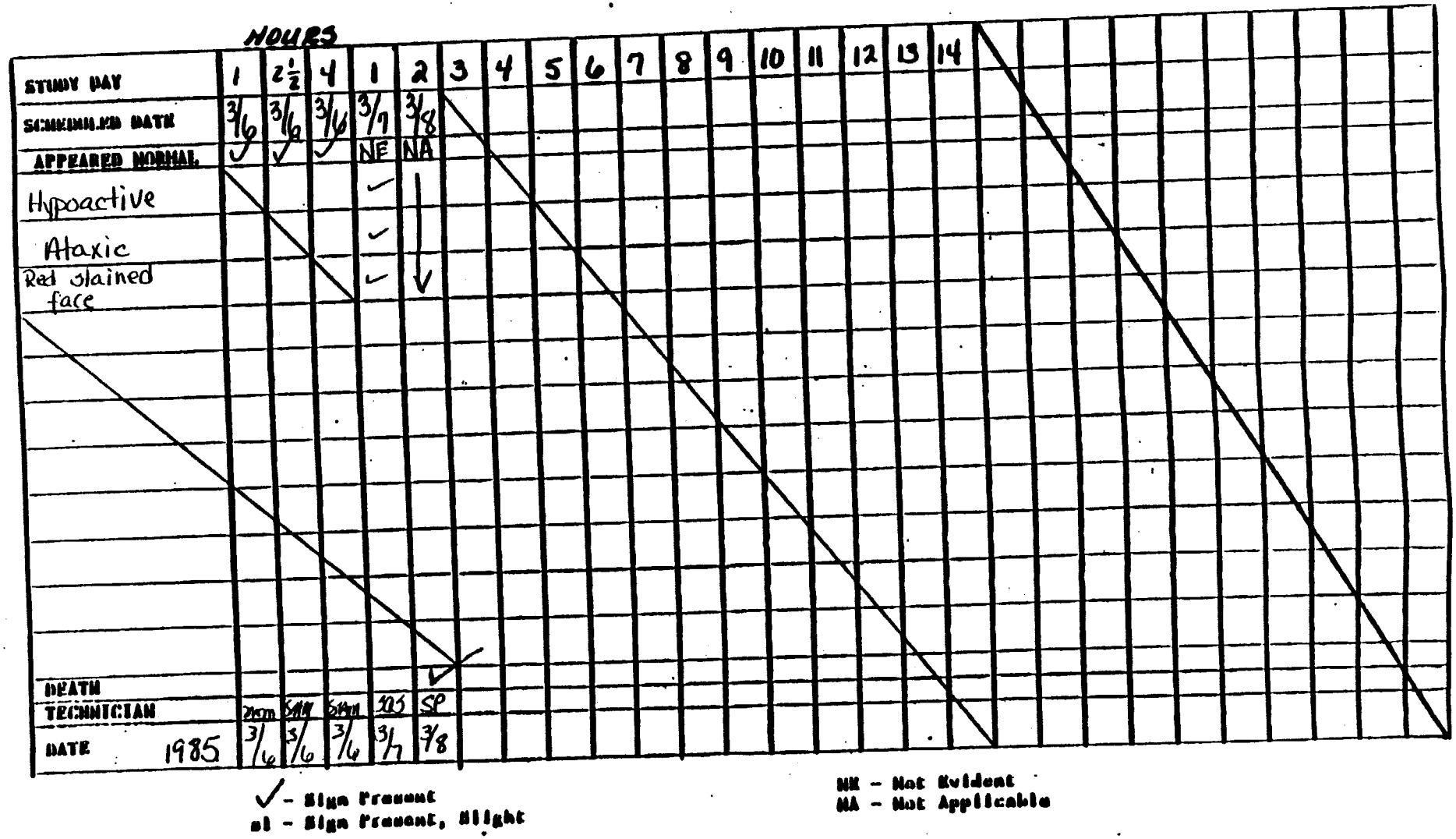
NE - Not Evident
NA - Not Applicable

001151

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473 SEX ♀

TEST MATERIAL: T-3727 DOSAGE LEVEL: 2.0g/kg ANIMAL/EAR TAG NO. C28693



✓ - Sign Present
sl - Sign Present, Slight

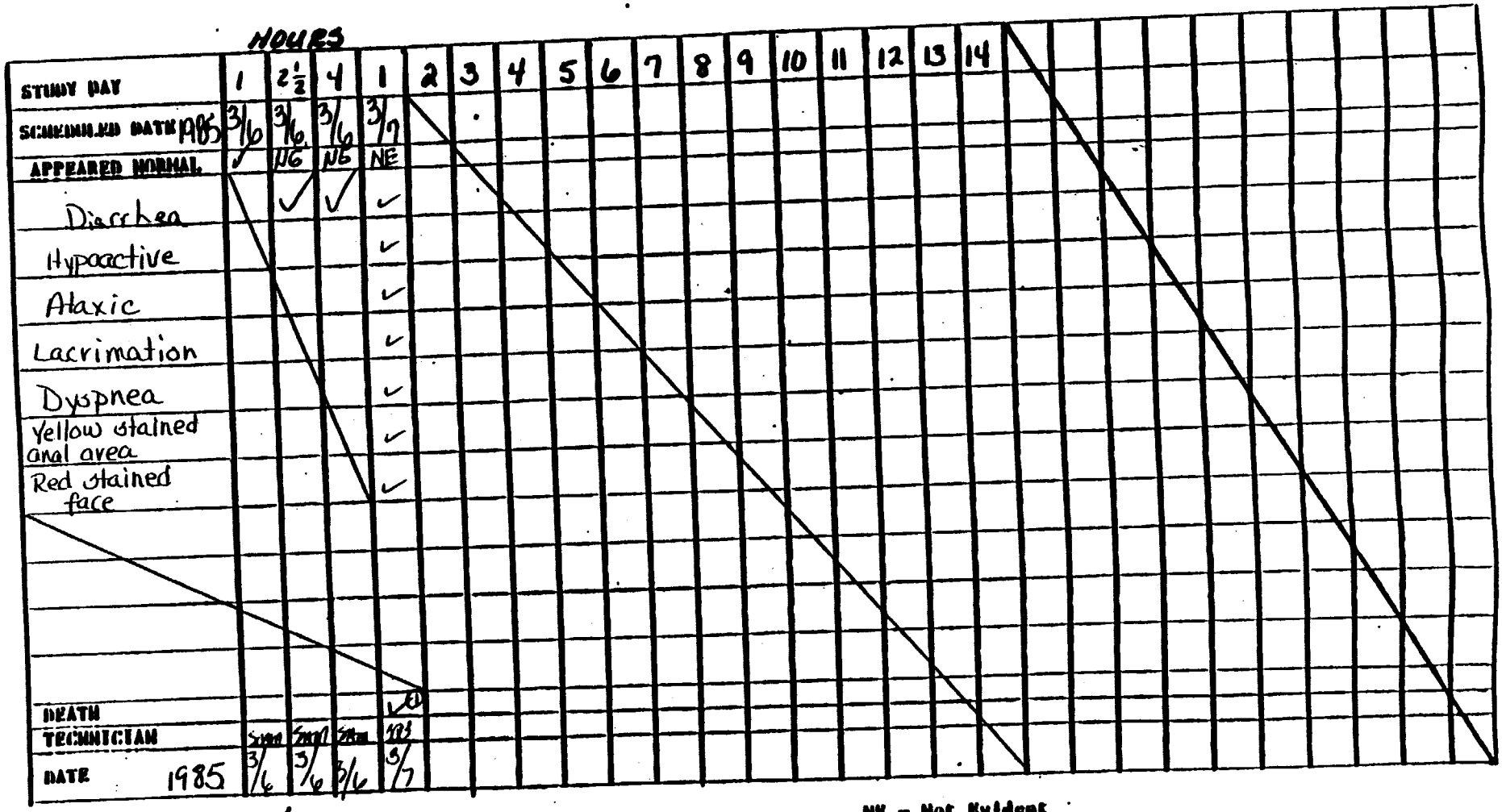
NE - Not Evident
NA - Not Applicable

C01152

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473 SEX ♀

TEST MATERIAL. T-3727 DOSAGE LEVEL. 2.00g/kg ANIMAL/EAR TAG NO. C29497



✓ - Sign Present
sl - Sign Present, Slight
NE - Not Evident
NA - Not Applicable

① Animal died in p.m. 3-7-85 CBS

C01153

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473 SEX ♀

TEST MATERIAL: T-3727 DOSAGE LEVEL: 2.0gm/kg ANIMAL/EAR TAG NO. C28695

STUDY DAY	HOURS 1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE 1985	3/6	3/6	3/6	3/7	3/8												
APPEARED NORMAL	NE	NE	NE	NE	NA												
Diarrhea	✓	✓	✓	✓													
Hypoactive				✓													
Ataxic				✓													
Yellow stained abdomen & genital area				✓													
Red stained face				✓													
DEATH					✓												
TECHNICIAN	SM	SM	SM	MB	SP												
DATE 1985	3/6	3/6	3/6	3/7	3/8												

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

STUDY TITLE: Acute Oral Toxicity

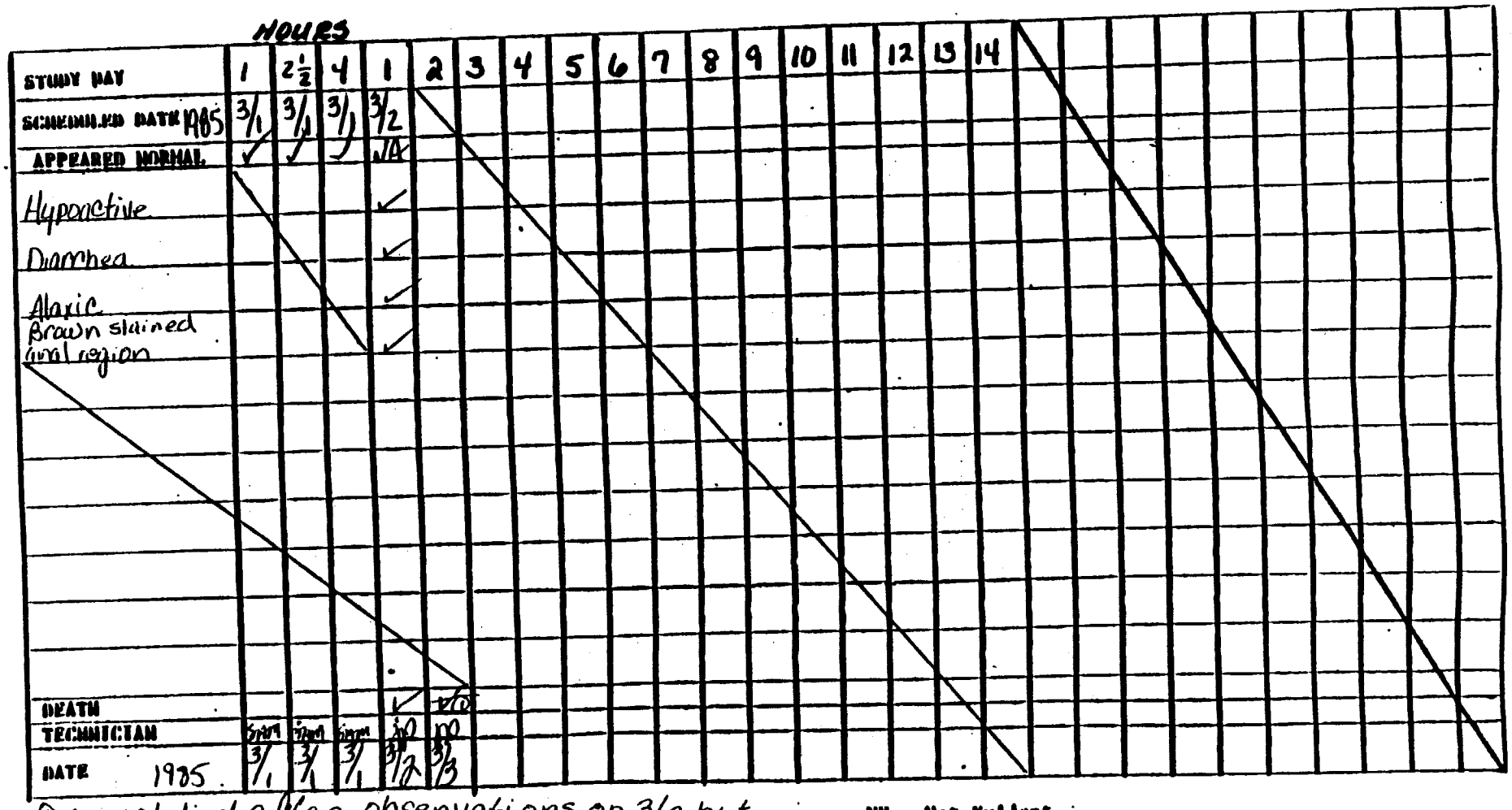
NT NO. 50202473

TEST MATERIAL: T-3727

SEX ♂

DOSAGE LEVEL: 5.00g/kg

ANIMAL/EAR TAG NO. C2-8545



① animal died after observations on 3/2 but not corrected or footnoted until 5-3-85 jp

✓ - Sign Present
I - Sign Present, Slight
NK - Not Evident
NA - Not Applicable

C01155

STUDY TITLE: Acute Oral Toxicity

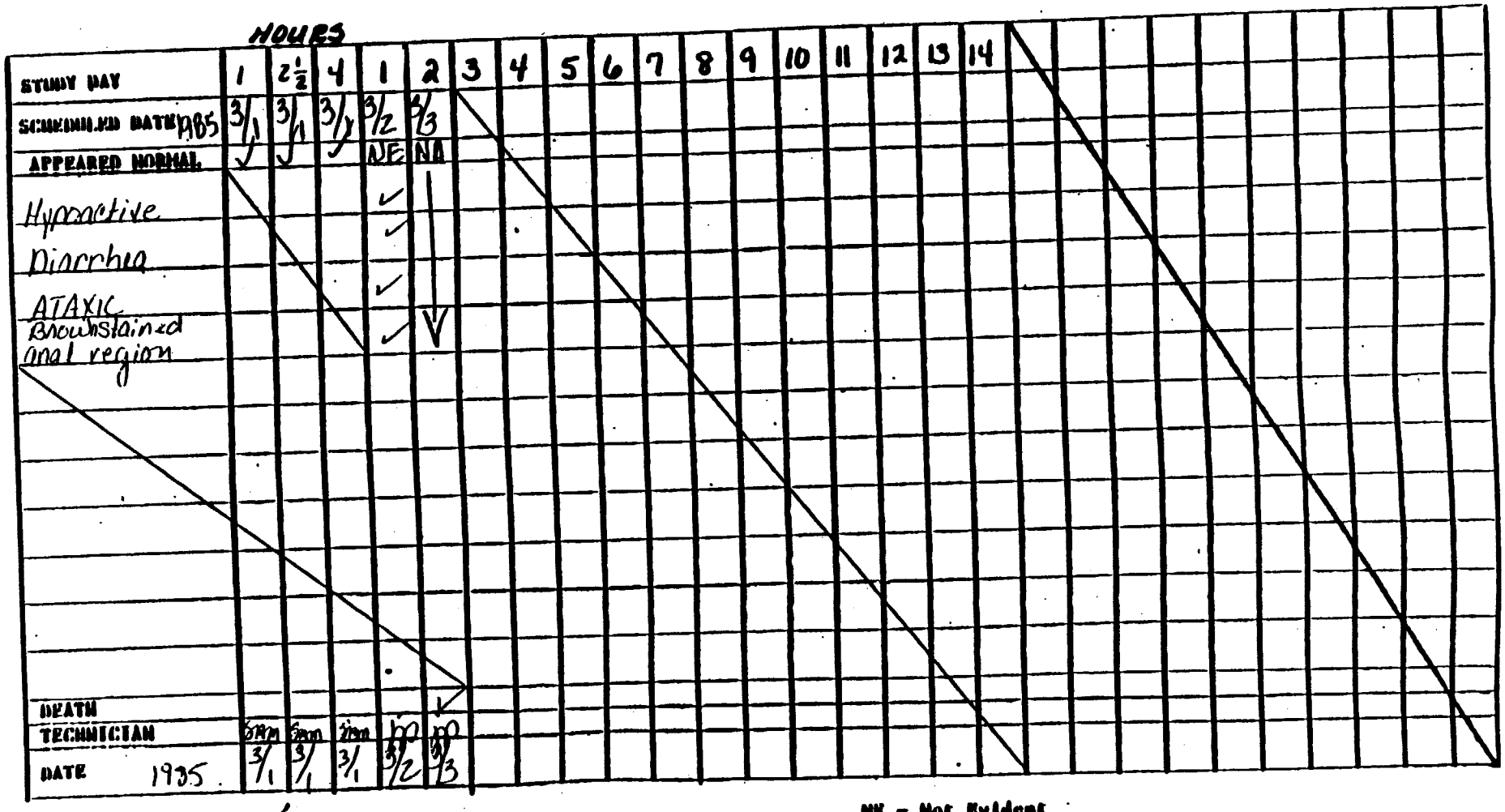
HT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♂

DOSAGE LEVEL: 5.00g/kg

ANIMAL/EAR TAG NO. C2-8456



C01156

STUDY TITLE: Acute Oral Toxicity

NT NO. 50202473

TEST MATERIAL T-3727

SEX ♂

DOSAGE LEVEL 5.00g/kg

ANIMAL/EAR TAG NO. C2-9314

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE ABS	3/1	3/1	3/1	3/2													
APPEARED NORMAL	✓	✓	✓	NA													
DEATH				↓													
TECHNICIAN	[illegible]	[illegible]	[illegible]	[illegible]													
DATE 1935	3/1	3/1	3/1	3/2													

✓ - Sign Present
sl - Sign Present, Slight

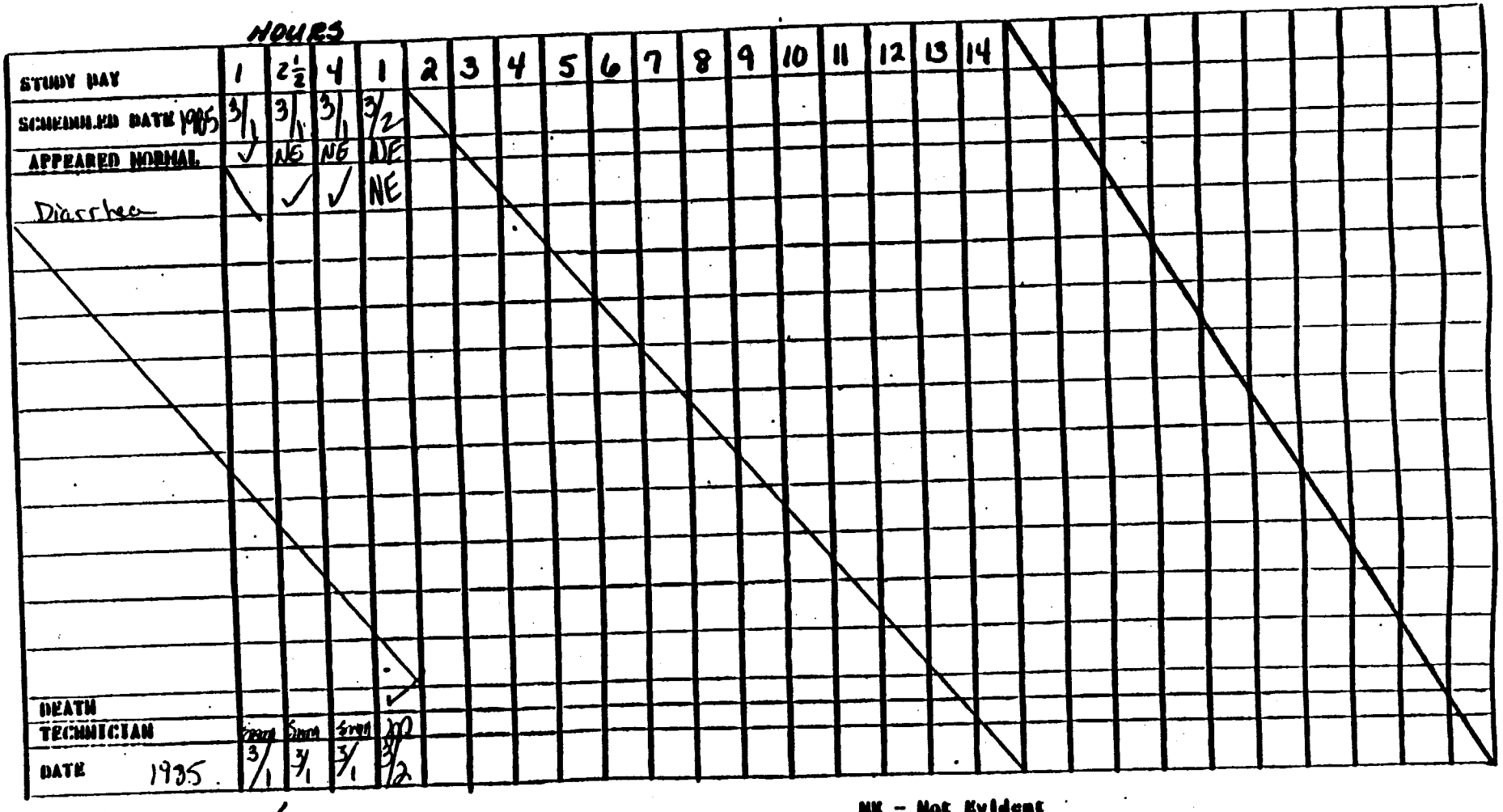
NK - Not Evident
NA - Not Applicable

C01257

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473 SEX ♂

TEST MATERIAL T-3727 DOSAGE LEVEL 5.00g/kg ANIMAL/EAR TAG NO. C2-8198



✓ - Sign Present
sl - Sign Present, Slight

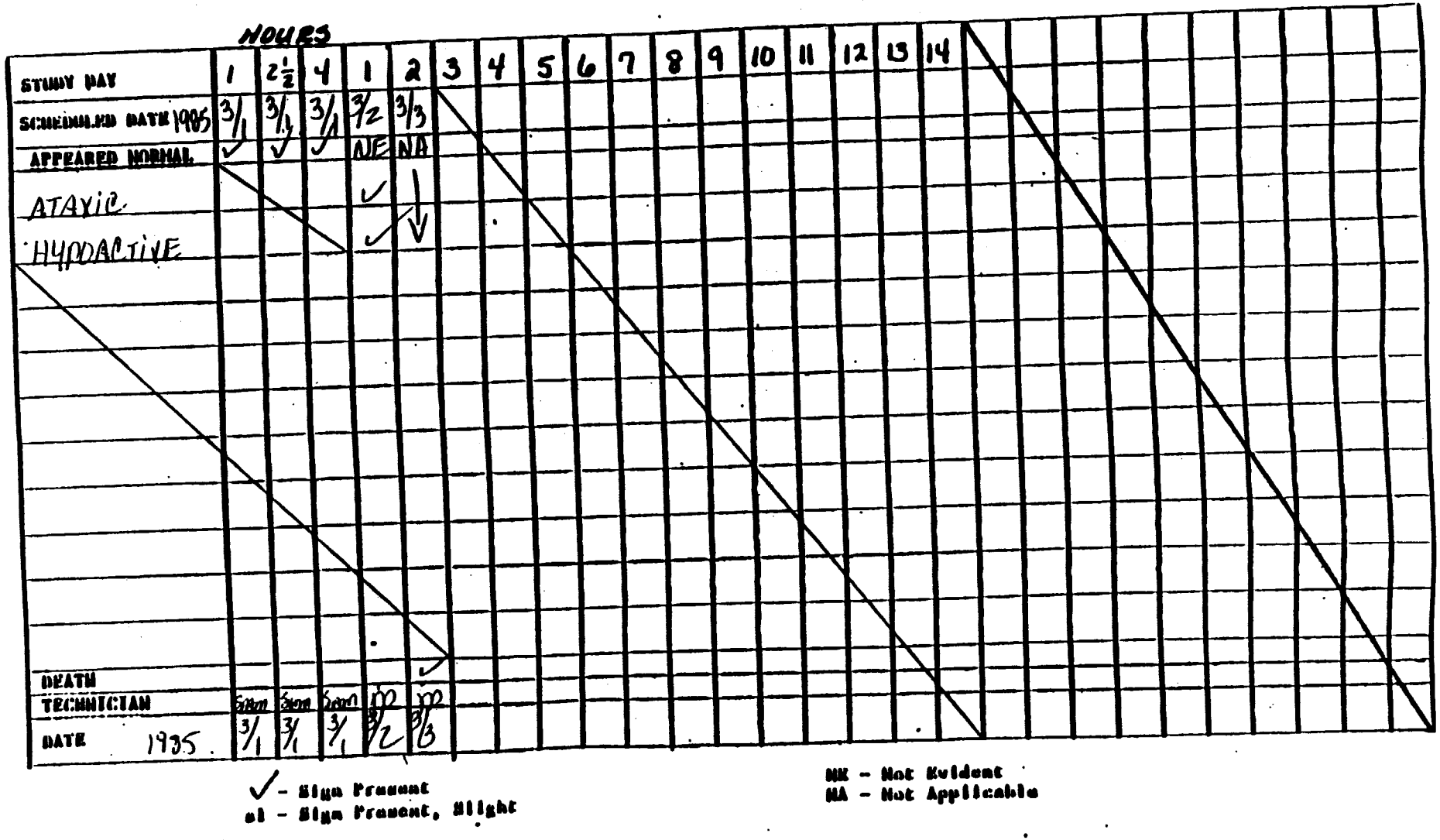
NE - Not Evident
NA - Not Applicable

C01158

STUDY TITLE: Acute Oral Toxicity

HT NO. 50202473 SEX ♂

TEST MATERIAL T-3727 DOSAGE LEVEL 5.0g/kg ANIMAL/EAR TAG NO. C2-9319



✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01159

STUDY TITLE: Acute Oral Toxicity

NT NO. 50203473

TEST MATERIAL. T-3727

SEX ♀

DOSAGE LEVEL. 5.00g/kg

ANIMAL/EAR TAG NO. C2-8498

HOURS

STUDY DAY	1	2½	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SCHEDULED DATE	3/1	3/1	3/1	3/2													
APPEARED NORMAL	NE	NE	NE	NA													
Hypoactive	✓	✓	✓	↓													
Dark Stained nose and mouth		✓	✓	↓													
DEATH				✓													
TECHNICIAN	am	am	am	[illegible]													
DATE 1995	3/1	3/1	3/1	3/2													

✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

G01160

STUDY TITLE: Acute Oral Toxicity

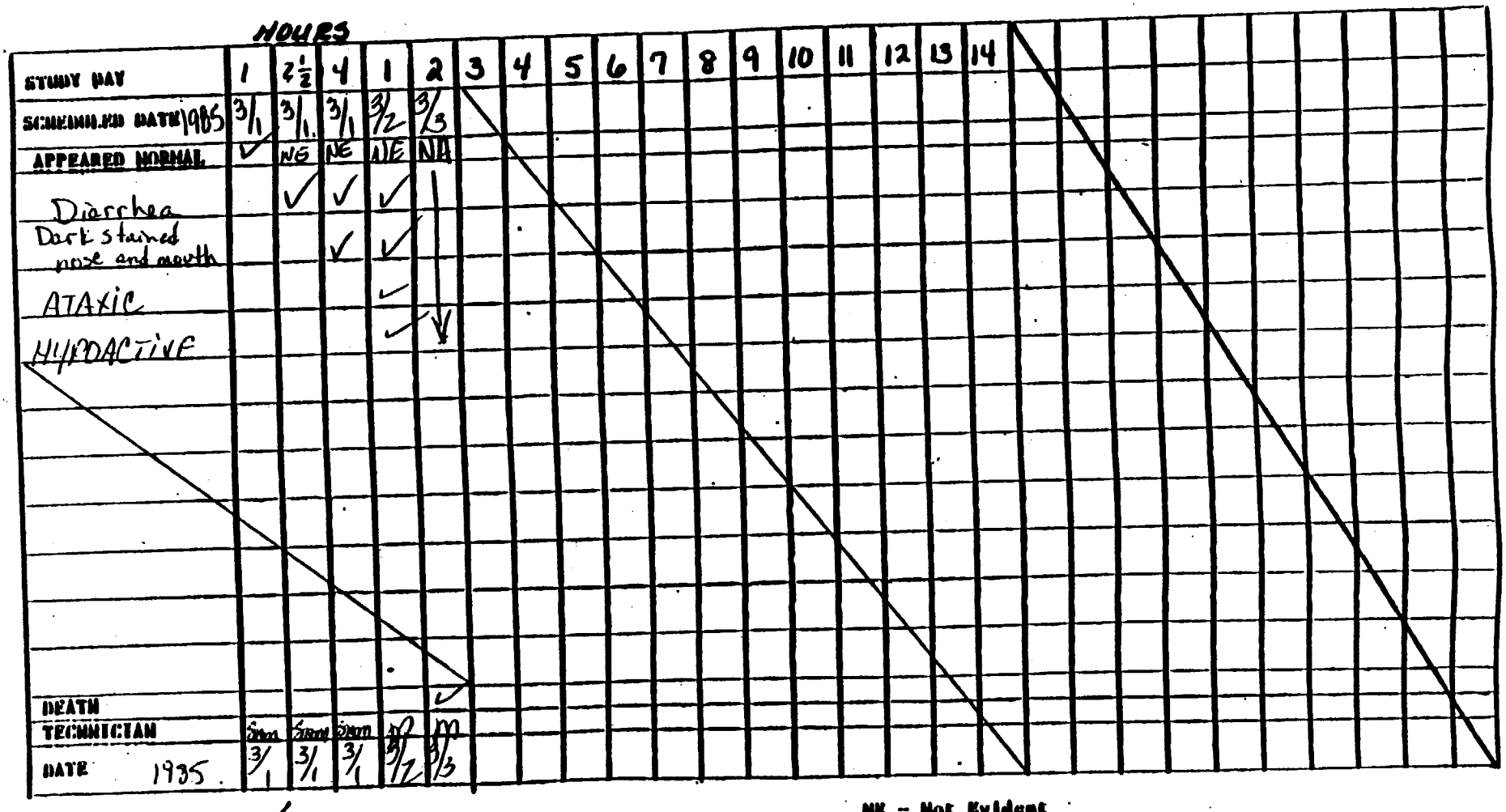
HT NO. 50202473

TEST MATERIAL: T-3727

SEX: ♀

DOSAGE LEVEL: 5.00 g/kg

ANIMAL/EAR TAG NO. C2-8499



✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01181

STUDY TITLE: Acute Oral Toxicity

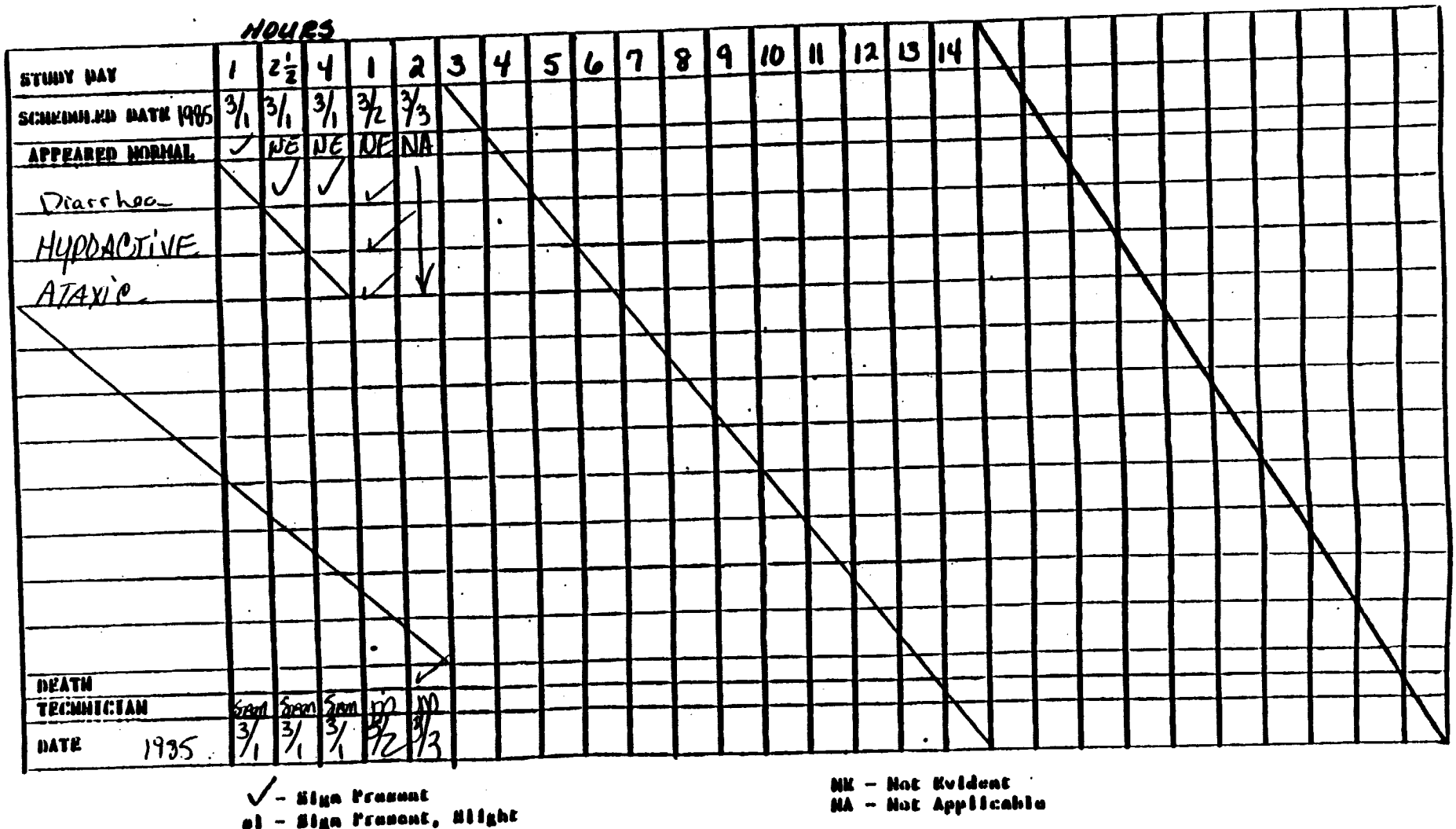
NT NO. 50202473

TEST MATERIAL. T-3727

SEX ♀

DOSAGE LEVEL. 5.00g/kg

ANIMAL/EAR TAG NO. C2-8500



✓ - Sign Present
sl - Sign Present, Slight

NK - Not Evident
NA - Not Applicable

C01162

STUDY TITLE: Acute Oral Toxicity

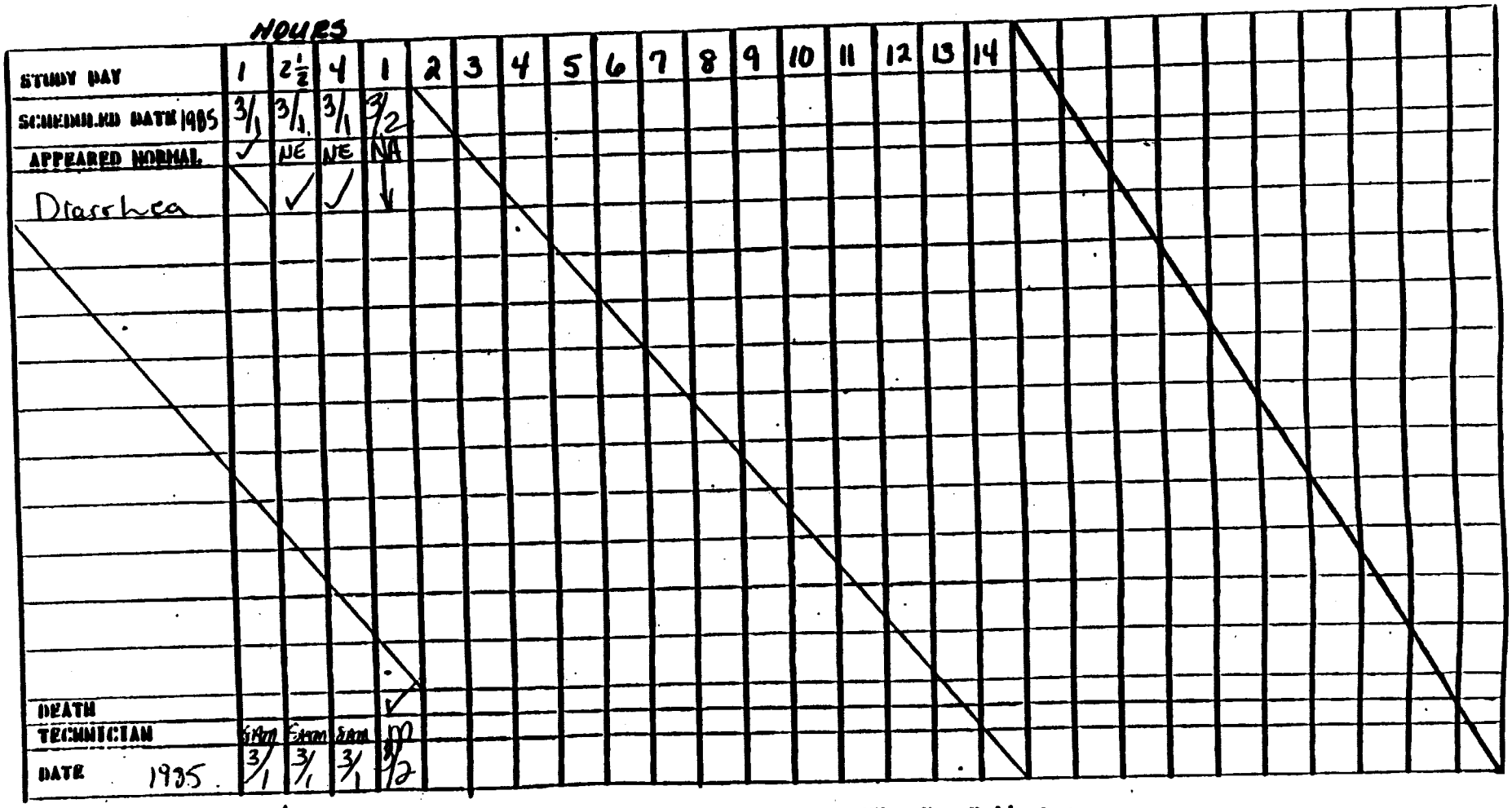
NT NO. 50202473

TEST MATERIAL. T-3727

SEX ♀

DOSAGE LEVEL. 5.00g/kg

ANIMAL/EAR TAG NO. C2-8501



✓ - Sign Present
sl - Sign Present, Slight

NE - Not Evident
NA - Not Applicable

C01163

STUDY TITLE: Acute Oral Toxicity

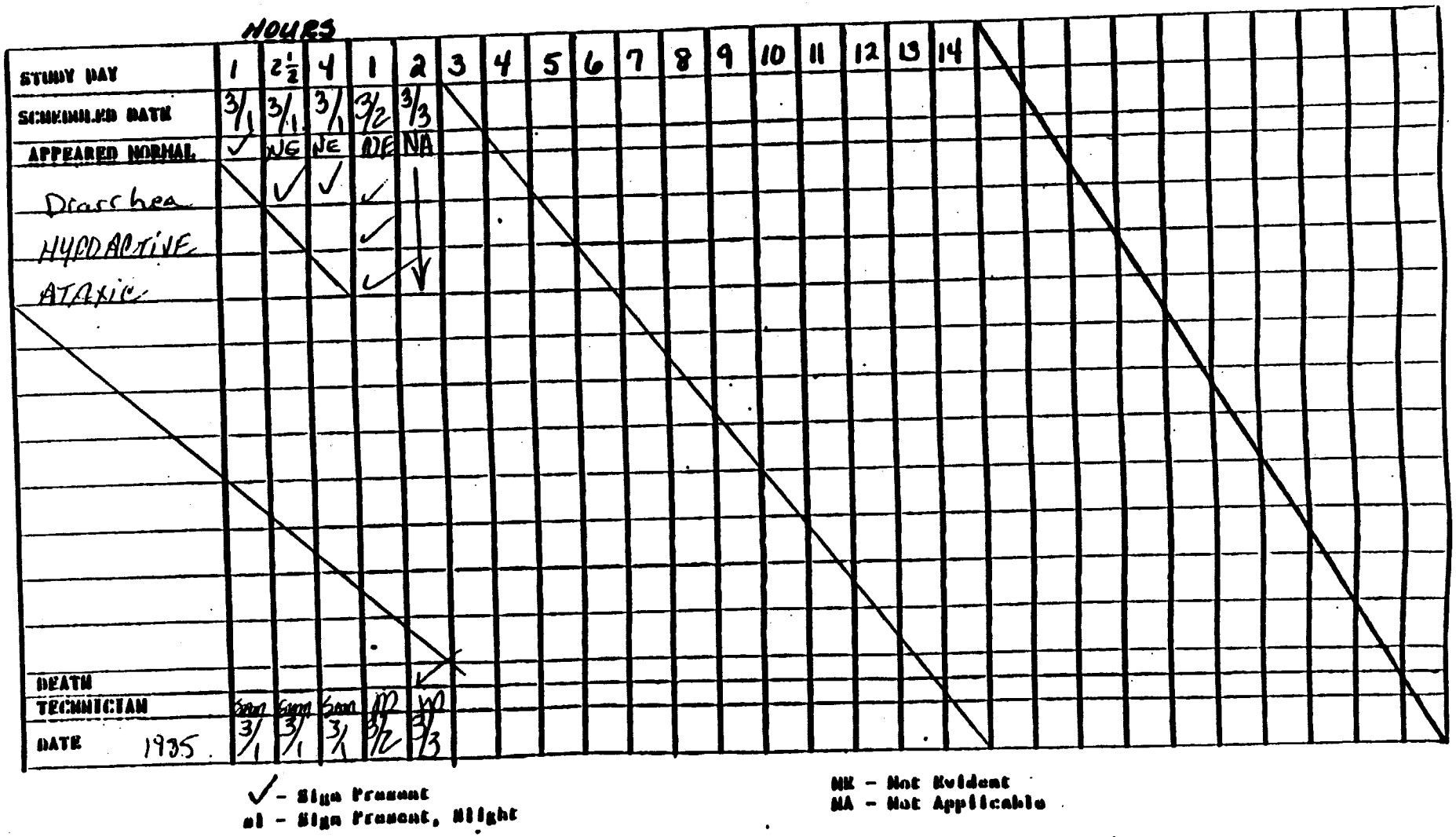
HT NO. 50202473

TEST MATERIAL T-3727

SEX ♀

DOSAGE LEVEL 5.00g/kg

ANIMAL/EAR TAG NO. C2-8503



✓ - Sign Present

sl - Sign Present, Slight

NE - Not Evident

NA - Not Applicable

PRIMARY SKIN IRRITATION SCORING SCALE

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

PRIMARY DERMAL IRRITATION STUDY

Test Compound: T-3727 30

HLA Number: 50202473

Dose: 0.5 ml/site

Vehicle: moistened with 0.9% saline

pH Result: NA

Source: Hazleton Research Products

Room Number: 161-1

Date Animals Received: 2-5-85

Date Animals Clipped: 2-28-85

Tech: CK

Initiated by: CK

Date: 3-1-85

Skin Preparation: intact

Reviewed by: MN

Date: 3-1-85

FO-		7819 ♀	7816 ♀	7800 ♀				Technician	Recorded by	1985 Date	Ktron Scale used:
Animal No./Sex								CK	CK	3-1	5921
Initial Body Weight (g)		2960	2840	3112							Dermal Irritation Score
Observation Period											
4 Hours	Erythema	0	0	0				CK	CK	3-1	0.0 SG
	Edema	0	0	0							
24 Hours	Erythema	0	0	0				MN	MN	3-2	0.0 SG
	Edema	0	0	0							
48 Hours	Erythema	0	0	0				MN	MN	3-3	0.0 SG
	Edema	0	0	0							
72 Hours	Erythema	0	0	0				CK	CK	3-4	0.0 SG
	Edema	0	0	0							
96 Hours	Erythema										
	Edema										
7 Days	Erythema										
	Edema										
7 Day Body Weight (g)											Scale used:

① Entry Error ✓ 3-1-85 CK

NA - not applicable.
A - subcutaneous hemorrhage.
B - blanching.
N - possible necrotic area.

Reviewed by: SG

Date: 3-4-85

(4251A)

C01166

PROTOCOL - APPENDIX I

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed	4*

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

* Starred figures indicate positive effect.

Primary Eye Irritation Test

Initial Sodium Fluorescein Exam and Animal Body Weights

Test Compound T-3727 RT No. 50202473

pH Result NA

Dose (g) 0.09 (0.1 ml equivalent) Room No. 161-1

Dose (ml) NA Dosed By MP Date 2/28/85

Date Animals Received 2-5-85 Reviewed By pgr Date 2/28/85

Source: Hazleton Research Products

Animal No.	Sex	Initial SF*	Vocalization Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
FO-7813	♀	NEG	N	2666			
7814	♀	NEG	N	2715			
7815	♀	NEG	N	3000			
TECHNICIAN	MP	(illegible)	MP	MP			
RECORDED BY	MP	(illegible)	MP	MP			
DATE	2/28 1985	2/27	2/28	2/28			
SCALE USED				KTron 5228			

* Sodium Fluorescein Examination
NEG = Negative
POS = Positive
NA = Not Applicable
Y = Yes
N = No

Time of dosing - 1:50 PM MP 2-28-85
Time of first observation - 2:50 PM MP 2-28-85

Primary Eye Irritation Test Observations

Test Compound T-3727 RT No. 50202473

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 1 hour

Animal No./ Ear Tag No. FO-	7813	7814	7815			
Location of Corneal Lesions Tail <——> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	1 INJ	0	0			
Conjunctivae -						
Redness	1	1	1			
Chemosis	1	1	1			
Discharge	1 B	1 B	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	MP	MP	MP			
Recorded By	MP	MP	MP			
Date 1985	2/28	2/28	2/28			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: SG Date: 3-4-85 Eye Irritation Score: 7.0 ✓ds
(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3727 RT No. 50202473

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 24 hours

Animal No./ Ear Tag No. FO-	7813	7814	7815			
Location of Corneal Lesions Tail <——> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	1 INJ	0	0			
Conjunctivae -						
Redness	1	1	0			
Chemosis	1	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	SAM	SAM	SAM			
Recorded By	SAM	SAM	SAM			
Date 1985	3/1	3/1	3/1			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: [illegible] Date: 3-4-85 Eye Irritation Score: 3.7 14h

(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3727 RT No. 50202473

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 48 hours

Animal No./ Ear Tag No. FO-	7813	7814	7815			
Location of Corneal Lesions Tail <——> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	1 INJ	0	0			
Conjunctivae -						
Redness	1	0	0			
Chemosis	1	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	jm	jm	~~DAP~~ jm			
Recorded By	jm	jm	jm			
Date 1985	3/2	3/2	3/2			

Delay error 3-2-85 jm

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: [illegible] Date: 3-4-85 Eye Irritation Score: 3 0 s/b ✓

(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3727 RT No. 50202473

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 72 hours

Animal No./ Ear Tag No. FO-	7813	7814	7815			
Location of Corneal Lesions Tail <——> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	1 INS	0	0			
Conjunctivae -						
Redness	1	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	Neg	Neg	Neg			
Technician	JM	JM	JM			
Recorded By	JM	JM	JM			
Date 1985	3/3	3/3	3/3			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

Reviewed By: SG Date: 3-4-85 Eye Irritation Score: 2.3 SG JSH

(1311A)

Primary Eye Irritation Test Observations

Test Compound T-3727 RT No. 50202473

Test Eye Right Group NA

NA Washed NA Seconds following instillation of test material, the test eye was washed with NA ml of NA for NA seconds ✓ Unwashed

OBSERVATION PERIOD: 96 hours

Animal No./ Ear Tag No. FO-	7813	7814	7815			
Location of Corneal Lesions Tail <——> Head	○	○	○	○	○	○
Ocular Structure						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	0			
Conjunctivae -						
Redness	0	0	0			
Chemosis	0	0	0			
Discharge	0	0	0			
Sodium Fluorescein Exam	NA	NA	NA			
Technician	CK	CK	CK			
Recorded By	CK	CK	CK			
Date 1985	3/4	3/4	3/4			

A = Purulent Discharge
B = Clear Discharge
C = Petite Hemorrhage
D = Blanching
INJ = Injected
NEG = Negative
POS = Positive

E = Corneal Epithelial Damage, Peeling
F = Corneal Epithelial Damage, Piling
G = Corneal Epithelial Damage, Pitting
H = Pannus
I = Corneal Neovascularization
NA = Not Applicable

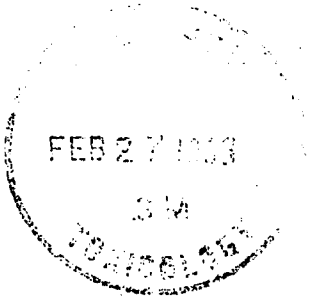
Reviewed By: SG Date: 3-4-85 Eye Irritation Score: 0.0 SG

(1311A)

HAZLETON LABORATORIES AMERICA, INC.

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WI 53707 • (608) 241-4471 • TLX 703956 HAZRAL MDS UD

February 25, 1985



Dallas D. Zimmerman, PhD
Manager, Toxicology Services International
3M Center
St. Paul MN 55144

Dear Dallas

Enclosed please find two copies each of the following protocols for sample T-3727, HLA No. 50202473:

Protocol No.	Study
TP-2069	Acute Oral Toxicity Study in Rats
TP-2072	Primary Eye Irritation Study in Rabbits
Tp-2071	Primary Dermal Irritation Study in Rabbits

These studies will be conducted in accordance with the OECD testing guidelines and GLP regulations.

Please sign all copies, retain one set for your file, and return the others to me. We can initiate these studies upon your verbal authorization.

Should you have any questions, please feel free to call.

Sincerely

Steven M. Glaza
Study Director
Acute Toxicology

SMG/mvh
Enclosures

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PROTOCOL TP2071

Primary Dermal Irritation Study in Rabbits (OECD Guidelines)

Study No. 50202473

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

February 25, 1985

PROTOCOL TP2071

Primary Dermal Irritation Study in Rabbits
(OECD Guidelines)

Study No.	50202473
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	T-3727
Sponsor's Representative	Dallas D. Zimmerman, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of February 25, 1985
Completion Date	Week of February 25, 1985
Final Report Date	Week of April 1, 1985

OBJECTIVE

The objective of this study is to determine the relative level of primary skin irritation of a test material on rabbits under semioccluded conditions. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 404, Acute Dermal Irritation/Corrosion, Adopted May 12, 1981[1] and the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Test Material:	T-3727.
Physical Description:	Off-white solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be discarded 30 days after issuance of the final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

TEST SYSTEM

Test Animal

Young adult albino rabbits of either sex of the New Zealand White strain, approximately 14 weeks of age, will be obtained from Hazleton Research

Products Inc., Denver, Pennsylvania. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. Historically, the New Zealand White albino rabbit has been the animal of choice for evaluating the effect of chemicals on the skin.

Acclimation

Upon receipt, the animals will be taken to a designated animal room where they will be acclimated for at least 1 week before being placed on test (OP-GENB 36). During acclimation, the animals will be examined for clinical abnormalities indicative of health problems (e.g., diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, evidence of injury, etc.). Any animals regarded as unsuitable for study purposes because of poor physical condition will not be released from acclimation and the reason(s) will be documented.

Identification

Each animal in the study will be assigned a permanent identification number and will be identified with a metal ear tag (OP-GENB 24). All data collected from an animal will be recorded and filed under its identification number.

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 21°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Teklad Laboratory Rabbit Diet. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

Study Design

Three rabbits will be selected at random based upon health and a body weight of 2.0-3.5 kg. Each animal will serve as its own control.

PROCEDURES

Preparation and Administration of Test Material

Twenty-four hours prior to test material administration, the hair will be clipped from the back and flanks of each animal. The treatment sites will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals.

The test material will be applied to the test area (approximately 6 cm^2) on each rabbit, in the amount of 0.5 g and will be moistened with deionized water. The treated area will be covered with a 2.5-cm x 2.5-cm gauze patch

secured with paper tape and loosely overwrapped with Saran Wrap and Elastoplast tape to provide a semiocclusive dressing. Collars will be used to restrain the animals during the 4-hour exposure period.

Reason for Route of Administration

Historically, the route of choice based on the method of Draize.[4]

Observations

After the 4 hours of exposure the patches and the test material will be removed as thoroughly as possible using water or an appropriate solvent without irritating the skin. Thirty minutes after removing the patches, the degree of erythema and edema will be recorded according to the Draize Technique (Attachment 1). Subsequent readings will be taken at 24, 48, and 72 hours after patch removal. Further observations may be recorded, as necessary, to establish reversibility. If irritation is increasing in severity at the 72-hour examination period, observations will be repeated at 96 hours and at 7 and 14 days, if applicable.

Body weights will be taken just prior to test material administration and at weekly intervals during the study. Observations and body weights will be recorded in the study notebook.

Pathology

All animals, whether dying on test or sacrificed at study termination, will be discarded.

Report

The final report will present a description of the test material, a description of the test system, dates of study initiation and termination, a tabulation of irritation data, and a description of any toxic effects other than dermal irritation.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. "Acute Dermal Irritation/Corrosion", OECD Guidelines for Testing Chemicals, Section 404, May 12, 1981.

2. 21 CFR 58

3. DHEW Publications No. (NIH) 78-23 (1978).

4. Draize, J. H., "Dermal Toxicity," Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 46-59 (1959).

PROTOCOL APPROVAL

Dallas D. Zimmerman — 2-25-85

Dallas D. Zimmerman, PhD
Sponsor's Representative
3M

Date

Steven M. Glaza — 2-25-85

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

Date

(1069S/jg)

ATTACHMENT I

PRIMARY SKIN IRRITATION SCORING SCALE

1. Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

2. Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

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PROTOCOL TP2069

Acute Oral Toxicity Study in Rats (OECD Guidelines)

Study No. 50202473

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

February 25, 1985

PROTOCOL TP2069

Acute Oral Toxicity Study in Rats
(OECD Guidelines)

Study No.:	50202473
Study Location:	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material:	T-3727
Sponsor's Representative:	Dallas D. Zimmerman, PhD
Study Director:	Steven M. Glaza
Proposed Timetable	
Starting Date:	Week of February 25, 1985
Completion Date:	Week of March 11, 1985
Final Report Date:	Week of April 1, 1985

OBJECTIVES

To determine the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, adopted May 12, 1981[1] and the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Test Material:	T-3727.
Physical Description:	Off-white solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be discarded 30 days of issuance of the final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

Disposal

All waste feed, animal wastes, pan liners, and carcasses resulting from this study will be disposed of in a high-temperature incinerator (U.S. Smelting Furnace Company, Belleville, Illinois).

TEST SYSTEM

Animal Model

Young adult male and female albino rats (approximately 7 weeks of age) of the Sprague-Dawley strain will be obtained from Harlan Sprague-Dawley, Madison, Wisconsin. Rats will be selected at random from healthy animals that had been acclimated at HLA for at least 1 week. An adequate number of extras will be purchased in order that no animal in obviously poor health is placed on test. The weight variation in animals used on test will not exceed ±20% of the mean weight (i.e., mean = 250 g, range = 200 to 300 g).

Reason for Species Selection

The rat is the animal classically used due to its small size, ready availability, and large amount of background data.

Identification

Each animal will be assigned an individual animal number and ear tag which will accompany data collected from that animal throughout the study (OP-GENB 24).

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 22°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be individually housed in screen-bottom stainless steel cages held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Purina Rat Chow®. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

PROCEDURES

Experimental Design

Initially, a single dose of 5.0 g/kg will be administered to 10 animals (five males and five females). If no test material-related mortality is produced at this level, no further testing is required. If any mortality occurs at the 5.0-g/kg dose level, at the Sponsor's request, three or four geometrically spaced dose levels may be added. Each dose level will consist of 10 animals (five males and five females). Animals will be assigned to groups according to HLA Standard Operating Procedure OP-TOX 42.

Test Material Preparation and Administration

The test material will be suspended in an appropriate vehicle. Individual dosages will be calculated based upon the animal's body weight taken just before administration of the test material and administered by gavage.

Justification of Route of Administration

This is the method for administering a known quantity of test substance and has been the route of choice historically.

Observations

The animals will be observed individually for clinical signs and mortality at 1.0, 2.5, and 4 hours after test material administration. The animals will be observed daily thereafter for at least 14 days for clinical signs and twice daily (morning and afternoon) for mortality. The duration of observations may be extended when considered necessary. The time of death will be recorded as precisely as possible.

Individual body weights will be recorded just prior to study initiation and at 7 and 14 days following test material administration and at death. Changes in body weight will be calculated and recorded when survival exceeds 1 day.

Pathology

All test animals, whether dying during the study or sacrificed at termination, will be subjected to a gross necropsy examination and abnormalities recorded.

Report

The final report will contain a description of the test material, a description of how the study was conducted, response data for clinical signs, mortality and body weights by sex, a discussion of the data, and gross pathology findings.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 401, Acute Oral Toxicity, adopted May 21, 1981.

2. 21 CFR 58.

3. DHEW Publications No. (NIH) 78-23 (1978).

PROTOCOL APPROVAL

Dallas D. Zimmerman, PhD
Sponsor's Representative
3M

Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

2-25-85
Date

(1064S/jg)

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PROTOCOL TP2072

Primary Eye Irritation Study in Rabbits (OECD Guidelines)

Study No. 50202473

for

3M
St. Paul, Minnesota

by

Hazleton Laboratories America, Inc.
Life Sciences Division
3301 Kinsman Boulevard
Madison, Wisconsin 53704

February 25, 1985

PROTOCOL TP2072

Primary Eye Irritation Study in Rabbits
(OECD Guidelines)

Study No.	50202473
Study Location	Hazleton Laboratories America, Inc. Life Sciences Division 3301 Kinsman Boulevard Madison, Wisconsin 53704
Test Material	T-3727
Sponsor's Representative	Dallas D. Zimmerman, PhD
Study Director	Steven M. Glaza
Proposed Timetable	
Starting Date	Week of February 25, 1985
Completion Date	Week of March 1, 1985
Final Report Date	Week of April 1, 1985

OBJECTIVE

The objective of this study is to determine the level of irritation produced following a single exposure of a test material to one eye of albino rabbits. All aspects of this study will conform to the Organisation for Economic Cooperation and Development's Guidelines for Testing Chemicals, Section 405, Acute Eye Irritation/Corrosion, Adopted May 12, 1981[1] and the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.[2] All procedures will be done according to Hazleton Laboratories America, Inc. (HLA) Standard Operating Procedures (SOPs) referenced in this protocol.

TEST MATERIAL

Identification

Test Material:	T-3727.
Physical Description:	Off-white solid.
Purity and Stability:	Sponsor has purity and stability determinations on file.
Storage Conditions:	Store at room temperature.
Test Material Retention:	Any unused test material will be discarded 30 days after issuance of final report.
Safety Precautions:	Laboratory personnel will take the normal necessary precautions in handling a substance of unknown toxicity. Laboratory clothing, latex gloves, safety glasses, and a particle mask approved for toxic dusts must be worn.

TEST SYSTEM

Test Animal

Young adult albino rabbits of either sex of the New Zealand White strain, approximately 14 weeks of age, will be obtained from Hazleton Research Products, Inc., Denver, Pennsylvania. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Acclimation

Upon receipt, the animals will be taken to a designated animal room where they will be acclimated for at least 1 week before being placed on test (OP-GENB 36). During acclimation, the animals will be examined for clinical abnormalities indicative of health problems (e.g., diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, evidence of injury, etc.). Any animals regarded as unsuitable for the study purposes because of poor physical condition will not be released from acclimation and the reason(s) will be documented.

Identification

Each animal in the study will be assigned a permanent identification number and will be identified with a metal ear tag (OP-GENB 24). All data collected from an animal will be recorded and filed under its identification number.

Housing and Maintenance

The following environmental conditions will be maintained in the animal room used for this study (OP-TARC 230).

- Temperature: 21°C ±2°
- Relative humidity: 50% ±20%
- Air change: At least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity will be monitored throughout the study. Variations from prescribed environmental conditions will be documented.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[3] Care will be taken to ensure that the animals are not disturbed for reasons other than data collection and routine maintenance. The animals will be housed individually in screen-bottom stainless steel cages (heavy gauge) held on racks, with absorbent pan liners in the urine- and feces-collecting pans. Pan liners will be changed at least three times each week.

Feed and water will be provided ad libitum. The diet will be Teklad Laboratory Rabbit Diet. No contaminants are expected to be present in the feed or water which would interfere and affect the results of the study.

Study Design

Three rabbits will be selected at random based upon health and a body weight of 2.0 to 3.5 kg.

PROCEDURES

Preparation and Administration of Test Material

The rabbits' eyes will be examined using fluorescein dye procedures within 24 hours prior to test material administration. Only animals with no sign of

corneal injury or eye abnormalities will be utilized. One eye of each animal will be treated with the test material and the other eye will serve as the untreated control.

Each rabbit will receive 0.1 g (or the weight equivalent of 0.1 mL) of solid test material. If necessary, the solid test materials will be finely ground into a dust or powder. The test material will be placed into the everted lower lid of the rabbit's eye. The upper and lower lids are then to be gently held together for 1 second before releasing to prevent loss of material. The eyes of the rabbits will remain unflushed for 24 hours following instillation of the test material. After 24 hours, a washout may be used if considered appropriate.

Reason for Route of Administration

Historically, the route of choice based on the method of Draize.[4]

Observations

The treated eyes of all animals will be examined for ocular irritation at 1, 24, 48, and 72 hours after treatment. If no irritation or injury is present at 72 hours, the study will be terminated. If irritation is present at 72 hours, additional observations will be made at 96 hours and at 7, 14, and 21 days. If at any of these time points there is no irritation, the study will be terminated. If injury is still present at 21 days, the Sponsor will be contacted to determine whether the study should continue or be terminated. After recording the 24-hour observations, sodium fluorescein may be used to aid in revealing possible corneal injury. Irritation will be graded and scored using the Draize technique (Attachment 1).[4] All eye abnormalities will be recorded.

All animals that have a damaged eye producing undue stress or discomfort will be sacrificed for humane reasons after consulting with the Sponsor.

Body weights will be recorded prior to test material administration and at weekly intervals throughout the study. Observations and body weights will be recorded in the study notebook.

Pathology

All animals, whether dying or sacrificed at study termination, will be discarded.

Report

The final report will present a description of the test material, a description of the test system, dates of study initiation and termination, a summary table showing the irritation data at each observation period, and any special observations that were recorded.

Maintenance of Raw Data and Records

Original data or copies thereof will be available at HLA to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data, as well as the final report, will be retained in the archives of HLA, Madison, Wisconsin (OP-GEN 44).

REFERENCES

1. "Acute Eye Irritation/Corrosion," OECD Guidelines for Testing Chemicals, Section 405 (May 12, 1981).

2. 21 CFR 58.

3. DHEW Publications No. (NIH) 78-23 (1978).

4. Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity, Association of Food and Drug Officials of the U.S., Topeka, Kansas, pp. 49-51 (1959).

PROTOCOL APPROVAL

Dallas D. Zimmerman, PhD
Sponsor's Representative
3M

2-25-85
Date

Steven M. Glaza
Study Director
Group Leader, Acute Toxicology
Hazleton Laboratories America, Inc.

2-25-85
Date

(1068S/jg)

PROTOCOL - ATTACHMENT 1

(1) Cornea

(A) Opacity - degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(B) Area of cornea involved

One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral conjunctivae only)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Acute Oral Toxicity Screen

with T-3065CoC

in Albino Rats

Experiment No.: 0981AR0145

Conducted At: Safety Evaluation Laboratory
Riker Laboratories, Inc.
St. Paul, Minnesota

Dates Conducted: April 2, 1981 to April 16, 1981

Conducted By: KDOMalley 5/8/81
K. D. O'Malley, BS Date
Advanced Toxicologist
Study Director

Reviewed By: 5/15/81
K. L. Ebbens, BS Date
Supervisor, Acute Toxicology

dc: M. T. Case
K. L. Ebbens
F. D. Griffith
W. C. McCormick

Summary

An acute oral toxicity screen with T-3065CoC was conducted from April 2, 1981 to April 16, 1981 using male and female albino rats ranging in body weight from 209-293 grams. The test material was administered by gastric intubation at a dosage level of 5,000 mg/kg body weight with mortalities of 3/10 noted from day 1 to day 5 post dose administration. Diarrhea, lethargy and hypoactivity were the untoward reactions which were noted from 120 minutes to day four and body weight gains were noted in all animals which survived the 14 day observation period. Necropsy of the animals performed upon termination of the study revealed no visible lesions while hemorrhage of the gastrointestinal tract and lungs were noted in the animals which died acutely. The LD50 of T-3065CoC appears to be greater than 5,000 mg/kg in fasted male and female rats.

Introduction

The objective of this study was to approximate the acute oral LD50 of T-3065CoC in fasted albino rats. This study is not regulated by the Food and Drug Administration's Good Laboratory Practice Regulation of 1978, although the standard operating procedures of this laboratory adhere to the general principals of this regulation. The raw data generated by the Study Director and the final report are stored in the conducting laboratory's archives.

Method and Results

Young albino rats[a] were used in this test. All animals were held under quarantine for several days prior to testing with only animals which appeared to be in good health and suitable as test animals at the initiation of the study used. The rats were housed in suspended, wire-mesh cages in temperature and humidity controlled rooms and permitted a standard laboratory diet[b] plus water ad libitum except during the 16 - 20 hour period immediately prior to gastric intubation when food was withheld.

Five male and five female rats were administered the test material at a preselected dosage level. All doses were administered at a constant volume of 10 ml/kg directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubating needle[c].

After gastric administration of the test article, the rats were returned to their cages and observed for the following 14 days. Initial and final body weights, mortalities (Table 1) and adverse reactions (Table 2) were recorded. A necropsy was conducted on all animals that died during the study as well as those euthanatized at the end of the 14 day observation period (Table 1). The protocol, principal personnel involved in the study, composition characteristics, and Quality Assurance statement are contained in Appendices I - IV.

[a] Charles River Breeding Laboratories, Inc., Wilmington, MA
[b] Ralston Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri
[c] Popper and Sons, Inc., New Hyde Park, New York

TABLE 1

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3065CoC

Mortality, Necropsy and Body Weight Data

Dose [a] (mg/kg)	Sex	Animal Number	Individual Body Weights (g) Test Day Number: 0	14	Number Dead / Number Tested	Percent Dead
5,000	M	1R2607	274	(5 Days)	3/5	60
		1R2608	293	377		
		1R2609	289	(1 Day)		
		1R2610	279	373		
		1R2611	287	(2 Days)		
5,000	F	1R2590	235	274	0/5	0
		1R2591	241	281		
		1R2592	231	275		
		1R2593	233	275		
		1R2594	209	251		

[a] The test article was administered as a suspension in cottonseed oil.

The approximate oral LD50 appears to be greater than 5,000 mg/kg in fasted male and fema albino rats.

Necropsy

Necropsies performed upon termination of the study revealed no visible lesions, however, necropsy of the animals which died acutely revealed hemorrhagic gastrointestinal tracts and hemorrhagic lungs (one incidence).

TABLE 2

ACUTE ORAL TOXICITY SCREEN - ALBINO RATS

with T-3065CoC

Summary of Reactions

Dose mg/kg	Reactions / Sex	Observation Periods: Number Affected/Number Dosed — Minutes 1-30	Minutes 60	Minutes 120	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14
5000	F																	
	Hypoactivity	-	-	-	5/5	5/5	0/5	-	-	-	-	-	-	-	-	-	-	-
	Diarrhea	-	-	-	5/5	0/5	-	-	-	-	-	-	-	-	-	-	-	-
5000	M																	
	Hypoactivity	-	-	-	4/4	3/3	1/3	0/3	-	-	-	-	-	-	-	-	-	-
	Diarrhea	-	-	2/5	4/5	0/3	-	-	-	-	-	-	-	-	-	-	-	-
	Lethargy	-	-	-	1/4	0/3	-	-	-	-	-	-	-	-	-	-	-	-

No significant reaction (-)

GD1206

APPENDIX I
PROTOCOL

TEST: Acute Oral Toxicity
SPONSOR: 3M Commercial Chemicals Division
CONDUCTED BY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota
TEST ARTICLE: T-3065CoC
CONTROL ARTICLE: None
PROPOSED STARTING/COMPLETION DATE OF TEST: 3/81 3/81
TEST SYSTEM AND SOURCE: Rat, Charles River Breeding Laboratories, Inc., Wilmington, MA
Sex: M,F
Number: 5,5
Weight Range: 200-300 gm.

OBJECTIVE: The objective of this test will be to characterize the acute oral toxicity of the test article in albino rats. Rats were selected as a test system for reproducibility of response, historical use, ease in handling and general availability.

METHOD: The animals will be housed in stainless steel suspended wire mesh cages in temperature and humidity controlled rooms during both the quarantine and test periods, with food[a] and water offered ad libitum[b]. Each animal will be identified by color coding, according to the laboratory's standard operating procedure, which will correspond to a card affixed to the outside of the cage. A single dosage of 5,000 mg/kg will be administered each animal, however, if this dosage level does not adequately characterize the toxicity of the test article, additional animals will be administered the test article at supplemental dosage levels. Any additional dosage levels will be documented and filed with this protocol. The test article will be administered to the animals in the form received from the sponsor. After administration of the test article, the animals will be returned to their cages and observed for any untoward behavioral reactions for the following 14 days. Initial and final body weights will be recorded. A gross necropsy which will include, but not be limited to, heart, lungs, liver, kidneys and general gastrointestinal tract will be conducted on all animals which die during the conduct of the test as well as the animals surviving the test period. Any gross abnormalities which are observed during the conduct of the necropsy will be recorded with specific mention to the organ and/or site observed. The acute median lethal dose (LD50) of the test article will be calculated, if possible, using a probit analysis method at the end of the observation period. All raw data and the final report will be stored in the Riker Laboratories Archives, St. Paul, Minnesota.

[a] Purina Laboratory Chow, Ralston Purina, St. Louis, Missouri

[b] except during a 16-20 hour period immediately prior to dosing when food will be withheld.

W. Dean Thurmond 3/24/81 — Sponsor — Date
V.D. Miller 3/31/81 — Study Director — Date

Form 19171-16-PWO

APPENDIX II

Principal Participating Personnel Involved in the Study

Name	Function
G. E. Hart	Laboratory Technician Acute Toxicology
K. D. O'Malley, BS	Advanced Toxicologist Study Director
K. L. Ebbens, BS	Supervisor Acute Toxicology
G. C. Pecore	Supervisor Animal Laboratory

APPENDIX III

Composition Characteristics

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore information pertaining to composition characteristics is not applicable for inclusion in this study.

APPENDIX IV

Quality Assurance Statement

This study is not regulated by the Good Laboratory Practice Regulation of 1978 and therefore a statement signed and prepared by the Quality Assurance group is not applicable. This study was, however, audited by the Quality Assurance group.

In addition to the data audit, different significant phases for studies underway in the Toxicology Laboratory are inspected weekly on a recurring cycle, and the facilities are examined by Laboratory Quality Assurance on a three month schedule.

SRI International

IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY'S COMPOUND T-3752

Final Report

June 1985

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SUMMARY

SRI International examined 3M Company's Compound T-3752 for mutagenic activity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. Compound T-3752 was also screened for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay. Both assays were performed in the presence and absence of a rat-liver metabolic activation system. All tests were performed in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice Standards.

Compound T-3752 was reproducibly nonmutagenic and nonrecombinogenic when tested according to these procedures.

CONTENTS

QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, In Vitro Microbiological Mutagenecity Assays Of Compound T-3752, for the 3M Company.

Inspections and audits were performed during different phases of the study, and the Study Director and SRI management were notified of the findings of the Quality Assurance Unit.

This report accurately describes the methods and standard operating procedures of the study. Any deviations from the approved protocol were made with the proper authorization and documentation. Any deviations from standard operating procedures were documented.

Manager of Regulatory Affairs
and Quality Assurance

Date

INTRODUCTION

SRI International examined 3M Company's Compound T-3752 for mutagenicity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. Compound T-3752 was also tested for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay. An Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system was included in the assay procedures to provide metabolic steps that the microorganisms either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with S. typhimurium has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic. The assay procedure with S. cerevisiae is about 60% reliable in detecting carcinogens as agents that increase mitotic recombination. However, because the assay systems do not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response conclusively proves that a chemical is carcinogenic or noncarcinogenic to man.

Evaluation of experimental results from the Salmonella assay consists of comparing the number of histidine-independent colonies on the treated agar plates with the number observed on the control plates. Because all the plated Salmonella indicator organisms undergo a few cell divisions in the presence of the test chemical, the test is semiquantitative in nature. The plate test procedure does not permit quantitative determination of the number of cells surviving the chemical treatment. It is the demonstration of a mutagenic dose-response relationship that is important in establishing mutagenicity.

The test chemicals are assayed at several dose levels within a non-toxic dose range--with the exception of the highest dose level, which sometimes exhibits toxicity. Toxicity is evidenced by several phenomena: clearing of the background bacterial lawn growth, formation of pinpoint colonies consisting of surviving cells, and a decrease in the number of revertant colonies below the spontaneous background.

A chemical is considered a mutagen in the Salmonella assay if it elicits a reproducible, dose-related increase in the number of histidine revertants per plate in one or more tester strains.

The yeast Saccharomyces cerevisiae D3 is a eukaryotic microorganism capable of detecting mitotic recombination, as expressed through a mutation leading to a defective enzyme in the adenine-metabolizing pathway, resulting in a red-pigmented colony. In this assay, the yeast cells are exposed to several concentrations of the test chemical, usually ranging from a concentration that results in no killing to one that causes 50% killing. Any concentration that induces 90% killing is considered toxic. When the number of genetically altered colonies per milliliter (yield) and the ratio of altered colonies to survivors (frequency) from the treated cells are unequivocally larger than those of the solvent-treated controls, we conclude that the exposure of the cells to the compound induces mitotic recombination. If this event is dose-related, the observation is termed a positive response.

The assays with Compound T-3752 were begun on 24 May 1985 and testing was completed on 14 June 1985. Copies of the final report will be kept in our files (Building M, Room 213) and in SRI's Records Center. The raw data will be retained in Building 205, Room 13, for one year after the laboratory notebook has been filled and then will be stored in SRI's Records Center. All that remains of Compound T-3752 will be kept for six months in our chemical storage room (Building M, Room 217) and then returned to 3M Company.

MATERIALS

- Test Article
  - Name: T-3752
  - Date Received: 15 May 1985
  - Description: Amber waxy solid
  - Storage Conditions: Stored at room temperature in a secondary container
  - Special Testing Conditions: None
  - Stability: Assured by Sponsor

- Indicator Organisms
  - Species: Salmonella typhimurium LT2; Saccharomyces cerevisiae
  - Strains: TA1535, TA1537, TA1538, TA98, and TA100 for S. typhimurium; D3 for S. cerevisiae
  - Source: Dr. Bruce Ames, University of California, Berkeley, for the Salmonella; Dr. F. K. Zimmermann, W. Germany, for the Saccharomyces

- Metabolic Activation

  Aroclor 1254-induced, rat liver S-9; SRI Batch F-5;
  ~ 31.5 mg/ml protein

  Animal Supplier: Simonsen Laboratories, Gilroy, California

- Negative (Solvent/Diluent) Control Material

  Dimethylsulfoxide (DMSO), CAS No. 67-68-5
  Date Opened: 3 and 8 May and 3 June 1985
  Expiration Date: 3 and 8 May and 3 June 1986, respectively
  Manufacturer: American Scientific Products, McGraw Park, IL
  Purity: 0.12%--H_2O (for all)
  Lot No.: 4948 KVLX (for all)

- Positive Control Chemicals

9-Aminoacridine, CAS No. 90-45-9
Manufacturer: Pfaltz and Bauer, Stamford, CT

2-Anthramine, CAS No. 613-13-8
Manufacturer: Sigma Chemical Co., St. Louis, MO

2-Nitrofluorene, CAS No. 607-57-8
Manufacturer: Aldrich Chemical Co., Milwaukee, WI

Sodium azide, CAS No. 26628-22-8
Manufacturer: Difco Laboratories, Detroit, MI

1,2:3,4-Diepoxybutane, CAS No. 1464-53-5
Manufacturer: Pfaltz and Bauer, Stamford, CT

Sterigmatocystin, CAS No. 10048-13-2
Manufacturer: Aldrich Chemical Co., Milwaukee, WI

- Counters Used

  - New Brunswick Scientific BioTran II® Automated Colony Counter, Model C111, SRI Nos. 0030 6126 00, 0030 0151 00, and 0012 3318 00

  - New Brunswick Scientific Bactronic® Colony Counter, Model C110, SRI Nos. 0030 1471 00, 0012 3108 00, and 0013 0788 00

METHODS

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The *Salmonella typhimurium* strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The *his*$^+$ revertants are easily visible as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased, usually in a dose-related manner.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley. In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrB*). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvrB* mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically induced DNA lesions and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to *his*$^+$ by many mutagens that cause base-pair substitutions. Strain TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect

the presence of the plasmid in the cell. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept frozen in nutrient broth supplemented with 10% sterile glycerol at -80°C in 1-ml aliquots containing about 10^9 cells. New frozen stock cultures are made every three months from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, the frozen 1-ml cell cultures are allowed to thaw at room temperature before inoculation in 50 ml of glucose minimal liquid medium supplemented with an excess of biotin and histidine. The cultures are grown at 37°C, unshaken for 4 hours, then gently shaken (100 rpm) for 11 to 14 hours. All strains are genetically analyzed whenever experiments are performed.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amine type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens. Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use. In brief, adult male Sprague-Dawley rats (200 to 250 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyls). This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed, sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed with an equal volume of cold, sterile 0.15 M KCl, minced with sterile surgical scissors in three volumes of 0.15 M KCl (3 ml/g of wet organ), and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 × g, and the supernatant, referred to as the S-9 fraction, is quickly frozen on dry ice and stored at -80 °C.

The metabolic activation mixture for each experiment consists of, for 50 ml:

- 5.0 ml of S-9 fraction
- 1.0 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.25 ml of glucose-6-phosphate (1 M)
- 2.0 ml of NADP (0.1 M)
- 25.0 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 16.75 ml of sterile H_2O.

The amount of S-9 fraction delivered to each plate is 50 µl.

Plate Incorporation Assay

Prior to testing, the test article is serially diluted from an initial stock. Generally, a preliminary experiment is conducted to find a suitable dose range for testing. The article is usually tested over a minimum of six dose levels, the highest nontoxic dose level being 10 mg/plate unless solubility, mutagenicity, or toxicity dictates a lower upper limit. When extracts are made, various undiluted aliquots are tested, usually over a dose range of 5 to 100 or 200 µl/plate. When liquids are tested, occasionally the sample is not diluted and various aliquots are used. All assays are repeated at least once on a separate day.

The plate incorporation assay is performed in the following way. To a sterile 13 × 100-mm test tube placed in a 43 °C heating block we add:

(1) 2.00 ml of 0.6% agar containing 0.6% NaCl, 0.05 mM biotin, and 0.05 mM histidine

(2) 0.05 ml of indicator organisms (about 10^8 bacteria)

(3) 0.05 ml of a solution of the test article

(4) 0.50 ml of metabolic activation mixture (if appropriate).

This mixture is stirred gently and then poured on plates containing about 25 ml of minimal glucose agar. After the top agar has set, the plates are incubated for 48 hours at 37°C. The number of $\underline{his}^+$ revertant colonies is counted using a BioTran II automated colony counter when possible. When accurate counts cannot be obtained (e.g., because of precipitate), the plates are counted manually using an electric probe colony counter.

Concurrent sterility, negative (solvent/diluent), and positive controls are run with every experiment. Sterility controls include plating out separately steps (3) and (4). For negative controls, we use steps (1), (2), (4), and 0.05 ml of the solvent/diluent used for the test article, if appropriate. For positive controls, we test each bacterial culture using steps (1), (2), (3), and (4) with the following mutagens:

- Sodium azide for the base-pair substitution mutants TA1535 and TA100
- 9-Aminoacridine for the frameshift mutant TA1537
- 2-Nitrofluorene for the frameshift mutants TA1538 and TA98
- 2-Anthramine for all tester strains, in the presence of metabolic activation.

We routinely check for true revertant ($\underline{his}^+$) colonies by replica plating from the parent to minimal glucose agar plates supplemented with biotin.

Criteria for Interpretation

Positive. A test article is considered a mutagen when it produces a reproducible, dose-related increase in the number of revertants in one or more strains. This increase should occur for at least three dose levels.

Negative. A test article is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments. The maximum dose level tested for nontoxic

compounds is 10 mg/plate (unless dictated otherwise by the sponsor or by solubility problems). For toxic compounds, only the highest dose level tested should show evidence of toxicity.

Inconclusive. When a test article cannot be identified clearly as a mutagen or nonmutagen in the standard plate assay, the results are classified as inconclusive.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enyzme in the adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various carcinogenic or recombinogenic agents. The recombinogenic activity of a compound or its metabolite is determined from the number of red-pigmented colonies appearing on test plates.

A culture of S. cerevisiae is stored at 4°C. For each experiment, broth containing 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.45% $(NH_4)_2SO_4$, 0.35% peptone, 0.5% yeast extract, and 2% dextrose is inoculated with a loopful of the stock culture and incubated overnight at 30°C, with shaking.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged and the cells are resuspended at a concentration of 10^8 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.0 ml of the resuspended culture
- 0.5 ml of either the metabolic activation mixture or buffer
- 0.2 ml of the test chemical
- 0.3 ml of buffer.

Several doses of the test chemical are tested (up to 5% w/v or v/v) in each experiment, and appropriate solvent/diluent controls are included. 1,2:3,4-Diepoxybutane without metabolic activation and sterigmatocystin with activation are used as positive controls.

The suspension mixture is incubated at 30°C for 4 hours on a roller drum. The sample is then diluted serially in sterile physiologic saline, and 0.2 ml of the 10^{-5} and 10^{-3} dilutions is spread on plates containing the same ingredients as the broth plus 2.0% agar; five plates are spread with the 10^{-3} dilution and three plates are spread with the 10^{-5} dilution. The plates are incubated for 3 days at 30°C, followed by at least 1 day at 4°C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates containing the 10^{-3} dilution are scanned with a dissecting microscope at 10× magnification, and the number of mitotic recombinants (red colonies or red sectors) is recorded. The surviving fraction of organisms is determined from the total number of colonies appearing on the plates of the 10^{-5} dilution.

Criteria for Interpretation

Positive. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter and in the relative number of mitotic recombinants per 10^5 survivors.

Negative. When no reproducible recombinogenic activity is obtained in any of the assays performed, the test results are considered to be negative.

Inconclusive. When a test article cannot be identified clearly as causing a positive or a negative response, the results are classified as inconclusive.

Statistical Analysis

No statistical analysis is performed for any of the assays. The results of the plate incorporation assay are a tabulation of the number of colonies appearing on the plates. The results of the *S. cerevisiae* D3 assay are tabulated after calculating the number of mitotic recombinants per 10^5 survivors. All calculations are expressed with two significant digits.

References

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RESULTS AND DISCUSSION

3M Company's Compound T-3752 was screened for mutagenic activity in the Ames Salmonella/microsome in vitro mutagenicity assay using the five standard strains of Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, and TA100. The assays were performed in duplicate, using three plates per dose, both in the presence and absence of a rat-liver metabolic activation system. DMSO was used as the solvent.

The microbial mutagenicity testing of this sample was performed twice at dose levels ranging from 10 to 5000 µg/plate (Tables 1 and 2). No increases in the number of revertant colonies over the spontaneous background were observed under any of the assay conditions used. Background lawn thinning was observed in the first assay only with strain TA1537 on all of the plates at 5000 µg/plate and on two of the three plates at 1000 µg/plate without activation. A light precipitate was noted at 5000 µg/plate; therefore, these plates were hand-counted. The results are presented in Tables 1 and 2.

Compound T-3752 was also screened for recombinogenic activity in the yeast Saccharomyces cerevisiae D3 assay for mitotic recombination. The assays were performed twice on separate days, both in the presence and absence of a rat-liver metabolic activation system. DMSO was used as the solvent. Since no toxicity was seen in the range-finding assay, the first experiment was performed at dose levels of 0.05, 0.1, 0.5, 1.0 and 5.0% (w/v). No increases in the number of mitotic recombinants were observed. The confirmatory assay was performed under conditions identical to those used in the initial assay. Again, no recombinogenic response was observed. The results of these two experiments are presented in Tables 3 and 4.

In conclusion, Compound T-3752 was reproducibly nonmutagenic and nonrecombinogenic when tested according to the procedures outlined in this report.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3752

Experiment Date: 28 May 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538			TA98			TA100*		
Negative Control																	
DMSO	–	50 µl	19	22	27	6	7	3	22	29	15	19	26	28	117	104	108
	+	50	8	16	6	3	9	10	23	17	39	31	35	17	130	122	135
Positive Controls																	
Sodium azide	–	1 µg	372	280	386										342	321	346
9-Aminoacridine	–	50				175	165	170									
2-Nitrofluorene	–	5							1686	1329	1284	818	883	848			
2-Anthramine	–	1							18	33	28	19	31	21	148	112	135
	+	1							230	214	808	97	109	355	230	280	330
	–	2.5	28	18	17	13	5	11									
	+	2.5	158	177	61	31	33	25									
Compound T-3752	–	10 µg	17	16	24	10	3	8	24	18	17	20	14	19	148	122	143
	–	50	10	9	15	5	5	6	9	20	28	14	18	20	106	117	82
	–	100	15	14	22	5	7	6	14	15	20	13	21	27	122	137	105
	–	500	12	11	21	4	6	7	23	17	27	17	22	30	143	114	142
	–	1000	9	15	17	8†	2†	13	14	15	21	19	19	12	140	151	154
	–	5000‡	14	11	18	5†	7†	8†	15	24	15	21	17	16	106	89	93
	+	10	13	7	17	13	8	5	26	34	33	41	30	32	98	121	101
	+	50	12	8	3	13	12	7	22	30	31	39	33	28	87	117	138
	+	100	14	5	13	6	5	6	35	18	36	31	27	34	109	78	85
	+	500	10	3	7	4	9	6	14	31	18	36	33	29	125	126	86
	+	1000	13	13	8	9	11	8	28	23	22	32	24	29	126	132	96
	+	5000‡	8	6	9	7†	5†	9†	28	35	27	43	39	50	93	97	124

*Experiment performed on 14 June 1985.
†Background lawn thinning.
‡Precipitated at this dose level; hand-counted.

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3752

Experiment Date: 6 June 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538*			TA98*			TA100		
Negative Control																	
DMSO	-	50 µl	31	37	27	3	10	3	16	15	18	20	17	23	160	129	123
	+	50	18	14	13	9	9	8	30	24	23	38	24	18	136	114	122
Positive Controls																	
Sodium azide	-	1 µg	562	586	621										600	666	636
9-Aminoacridine	-	50				456	307	390									
2-Nitrofluorene	-	5							1111	1017	842	675	452	502			
2-Anthramine	-	1							24	18	20	23	17	34	173	174	127
	+	1							202	160	256	109	108	194	306	336	311
	-	2.5	33	30	38	13	13	12									
	+	2.5	214	182	177	51	59	49									
Compound T-3752	-	10 µg	42	24	34	9	10	12	21	17	11	15	24	13	117	137	136
	-	50	32	33	36	3	10	11	14	17	17	24	30	20	153	179	127
	-	100	33	27	45	13	9	4	15	13	8	28	38	26	148	140	150
	-	500	35	42	33	6	9	8	19	11	17	23	18	14	140	152	130
	-	1000	31	33	31	6	8	10	7	13	12	10	25	14	143	126	122
	-	5000†	39	24	31	11	7	8	12	16	15	12	14	17	133	103	130
	+	10	16	11	10	13	7	12	18	16	19	25	20	29	174	108	136
	+	50	10	13	17	9	7	13	30	17	32	22	18	29	140	108	136
	+	100	10	11	20	12	3	17	23	24	36	22	24	23	153	128	146
	+	500	17	16	12	9	7	6	31	32	17	24	27	20	147	138	121
	+	1000	12	15	9	10	12	11	22	22	18	38	29	20	130	128	147
	+	5000†	18	18	18	15	11	18	37	35	33	28	17	28	150	101	130

*Experiment performed on 14 June 1985.

†Precipitated at this dose level; hand-counted.

Table 3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COMPOUND T-3752

Experiment Date: 24 May 1985

Compound	Metabolic Activation	Percent Concentration (w/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors*
Negative Control						
DMSO	-		7.3	100	4.5	6.2
	+		7.2	100	7.5	10
Positive Controls						
1,2:3,4-Diepoxy-butane	-	0.025	7.1	97	1331	1900
Sterigmatocystin	-	0.005	6.8	93	2	2.9
	+	0.005	7.4	100	303	410
Compound T-3752	-	0.05	7.2	99	9	13
	-	0.1	7.1	97	6	8.5
	-	0.5	6.9	95	7	10
	-	1.0	7.1	97	11	15
	-	5.0	7.6	100	5	6.6
	+	0.05	7.8	100	7	9.0
	+	0.1	6.4	89	9	14
	+	0.5	7.2	100	8	11
	+	1.0	6.0	83	6	10
	+	5.0	8.0	100	7	8.8

*All calculations are expressed to two significant figures.

Table 4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

COMPOUND T-3752

Experiment Date: 7 June 1985

Compound	Metabolic Activation	Percent Concentration (w/v)	Surviving Cells per ml ($\times 10^{-7}$)	Survivors (%)	Mitotic Recombinants per ml ($\times 10^{-3}$)	Mitotic Recombinants per 10^5 Survivors*
Negative Control						
DMSO	-		7.3	100	8	11
	+		6.9	100	10	14
Positive Controls						
1,2:3,4-Diepoxy-butane	-	0.025	7.3	100	1512	2100
Sterigmatocystin	-	0.005	7.3	100	8	11
	+	0.005	7.3	100	434	590
Compound T-3752	-	0.05	7.5	100	8	11
	-	0.1	7.0	96	9	13
	-	0.5	6.9	95	13	19
	-	1.0	6.9	95	6	8.7
	-	5.0	7.5	100	6	8.0
	+	0.05	7.2	100	6	8.3
	+	0.1	7.6	100	7	9.2
	+	0.5	7.1	100	9	13
	+	1.0	7.3	100	11	15
	+	5.0	7.7	100	11	14

*All calculations are expressed to two significant figures.

SRI International

IN VITRO MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY'S COMPOUND T-3727

Final Report

March 1985

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SUMMARY

SRI International examined 3M Company's Compound T-3727 for mutagenic activity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. The assay was performed in the presence and absence of a rat-liver metabolic activation system. All tests were performed in compliance with the United States Food and Drug Administration Good Laboratory Practice Standards.

Compound T-3727 was reproducibly nonmutagenic when tested according to these procedures.

CONTENTS

QUALITY ASSURANCE UNIT

Final Report Statement

SRI International assures the quality and integrity of this study, In-Vitro Microbiological Mutagenicity Assays of Compound T-3727, for the 3M Company.

The study was inspected on March 12, 1985 during the colony counting phase. The findings of the Quality Assurance Unit inspection were reported at the time of the inspection to the Study Director. SRI management was informed of the inspection results on March 12, 1985. A data audit was performed on March 25, 1985. The Study Director and SRI management were informed of the audit results on March 25, 1985.

The final report was audited and reviewed on March 25, 1985. The results of the final report review were communicated to the Study Director and SRI management on March 25, 1985. The final report accurately describes the methods and standard operating procedures and reflects the raw data of the study. Any deviations from the approved protocol and standard operating procedures were made with proper authorization and documentation.

Sherry J. Hansen

Quality Assurance Director

March 25, 1985

Date

INTRODUCTION

SRI International examined 3M Company's Compound T-3727 for mutagenicity in the standard Ames Salmonella/microsome assay with strains TA1535, TA1537, TA1538, TA98, and TA100 of the bacterium Salmonella typhimurium. An Aroclor 1254-stimulated, rat-liver homogenate metabolic activation system was included in the assay procedure to provide metabolic steps that the microorganisms either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with S. typhimurium has proven to be 80 to 90% reliable in detecting carcinogens as mutagens, and it has about the same reliability in identifying chemicals that are not carcinogenic. However, because the assay systems do not always provide 100% correlation with carcinogenicity investigations in animals, neither a positive nor a negative response conclusively proves that a chemical is carcinogenic or noncarcinogenic to man.

Evaluation of experimental results from the Salmonella assay consists of comparing the number of histidine-independent colonies on the treated agar plates with the number observed on the control plates. Because all the plated Salmonella indicator organisms undergo a few cell divisions in the presence of the test chemical, the test is semiquantitative in nature. The plate test procedure does not permit quantitative determination of the number of cells surviving the chemical treatment. It is the demonstration of a mutagenic dose-response relationship that is important in establishing mutagenicity.

The test chemicals are assayed at several dose levels within a nontoxic dose range--with the exception of the highest dose level, which sometimes exhibits toxicity. Toxicity is evidenced by several phenomena: clearing of the background bacterial lawn growth, formation of pinpoint

colonies consisting of surviving cells, and a decrease in the number of revertant colonies below the spontaneous background.

A chemical is considered a mutagen in the Salmonella assay if it elicits a reproducible, dose-related increase in the number of histidine revertants per plate in one or more tester strains.

The assays with Compound T-3727 were begun on 20 February 1985 and testing was completed on 27 February 1985. Copies of the final report will be kept in our files (Building M, Room 213) and in SRI's Records Center. The raw data will be retained in Building 205, Room 13, for one year after the laboratory notebook has been filled and then will be stored in SRI's Records Center. All that remains of Compound T-3727 will be kept for six months in our chemical storage room (Building M, Room 217) and then returned to 3M Company.

MATERIALS

- Test Article
  - Name: T-3727
  - Date Received: 19 February 1985
  - Description: Light-amber waxy solid
  - Storage Conditions: Stored at room temperature in a secondary container
  - Special Testing Conditions: None
  - Stability: Assured by Sponsor

- Indicator Organisms
  - Species: *Salmonella typhimurium* LT2
  - Strains: TA1535, TA1537, TA1538, TA98, and TA100 for *S. typhimurium*
  - Source: Dr. Bruce Ames, University of California, Berkeley

- Metabolic Activation

  Aroclor 1254-induced, rat liver S-9; SRI Batch F-4;
  ~ 26.5 mg/ml protein

- Negative (Solvent) Control Material

  Acetone, CAS No. 67-64-1
  Date Opened: 14 December 1984
  Expiration Date: 14 December 1985
  Manufacturer: American Scientific Products, McGraw Park, IL
  Purity: 99.7%
  Lot No.: KTEA

- Positive Control Chemicals

  9-Aminoacridine, CAS No. 90-45-9
  Manufacturer: Pfaltz and Bauer, Stamford, CT

  2-Anthramine, CAS No. 613-13-8
  Manufacturer: Sigma Chemical Co., St. Louis, MO

  2-Nitrofluorene, CAS No. 607-57-8
  Manufacturer: Aldrich Chemical Co., Milwaukee, WI

  Sodium Azide, CAS No. 26628-22-8
  Manufacturer: Difco Laboratories, Detroit, MI

- Counters Used

  - New Brunswick Scientific BioTran II® Automated Colony Counter, Model C111, SRI No. 0030 6126 00

  - New Brunswick Scientific Bactronic® Colony Counter, Model C110, SRI No. 0012 3108 00

METHODS

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The *Salmonella typhimurium* strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The *his*$^+$ revertants are easily visible as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased, usually in a dose-related manner.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley. In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrB*). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvrB* mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically induced DNA lesions and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to *his*$^+$ by many mutagens that cause base-pair substitutions. Strain TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for

a given dose of most mutagens. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cell. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept frozen in nutrient broth supplemented with 10% sterile glycerol at -80°C in 1-ml aliquots containing about 10^9 cells. New frozen stock cultures are made every three months from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, the frozen 1-ml cell cultures are allowed to thaw at room temperature before inoculation in 50 ml of glucose minimal liquid medium supplemented with an excess of biotin and histidine. The cultures are grown at 37°C, unshaken for 4 hours, then gently shaken (100 rpm) for 11 to 14 hours. All strains are genetically analyzed whenever experiments are performed.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of the aromatic amine type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens. Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use. In brief, adult male Sprague-Dawley rats (200 to 250 g) are given a single 500-mg/kg intraperitoneal injection of Aroclor 1254 (a mixture of polychlorinated biphenyls). This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food

is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed, sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed with an equal volume of cold, sterile 0.15 M KCl, minced with sterile surgical scissors in three volumes of 0.15 M KCl (3 ml/g of wet organ), and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 × g, and the supernatant, referred to as the S-9 fraction, is quickly frozen on dry ice and stored at -80°C.

The metabolic activation mixture for each experiment consists of, for 50 ml:

- 5.0 ml of S-9 fraction
- 1.0 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.25 ml of glucose-6-phosphate (1 M)
- 2.0 ml of NADP (0.1 M)
- 25.0 ml of sodium phosphate buffer (0.2 M, pH 7.4)
- 16.75 ml of sterile H_2O.

The amount of S-9 fraction delivered to each plate is 50 µl.

Plate Incorporation Assay

Prior to testing, the test article is serially diluted from an initial stock. In some cases, a preliminary experiment is conducted to find a suitable dose range for testing. The article is usually tested over a minimum of six dose levels, the highest nontoxic dose level being 10 mg/plate unless solubility, mutagenicity, or toxicity dictates a lower upper limit. When extracts are made, various undiluted aliquots are tested, usually over a dose range of 5 to 100 or 200 µl/plate. When liquids are tested, occasionally the sample is not diluted and various aliquots are used. All assays are repeated at least once on a separate day.

The plate incorporation assay is performed in the following way. To a sterile 13 × 100-mm test tube placed in a 43°C heating block we add:

(1) 2.00 ml of 0.6% agar containing 0.6% NaCl, 0.05 mM biotin, and 0.05 mM histidine

(2) 0.05 ml of indicator organisms (about 10^8 bacteria)

(3) 0.05 ml of a solution of the test article

(4) 0.50 ml of metabolic activation mixture (if appropriate).

This mixture is stirred gently and then poured on plates containing about 25 ml of minimal glucose agar. After the top agar has set, the plates are incubated for 48 hours at 37°C. The number of his$^+$ revertant colonies is counted using a BioTran II automated colony counter when possible. When accurate counts cannot be obtained (e.g., because of precipitate), the plates are counted manually using an electric probe colony counter.

Concurrent sterility, negative (solvent), and positive controls are run with every experiment. Sterility controls include plating out separately steps (3) and (4). For negative controls, we use steps (1), (2), (4), and 0.05 ml of the solvent used for the test article, if appropriate. For positive controls, we test each bacterial culture using the steps (1), (2), (3), and (4) with the following mutagens:

- Sodium azide for the base-pair substitution mutants TA1535 and TA100
- 9-Aminoacridine for the frameshift mutant TA1537
- 2-Nitrofluorene for the frameshift mutants TA1538 and TA98
- 2-Anthramine for all tester strains, in the presence of metabolic activation.

Statistical Analysis

No statistical analysis is performed. Results are a tabulation of the number of colonies appearing on the plates.

Criteria for Interpretation

Positive. A test article is considered a mutagen when it produces a reproducible, dose-related increase in the number of revertants in one or more strains. This increase must occur for at least three dose levels.

Negative. A test article is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments. The maximum dose level tested for nontoxic compounds is 10 mg/plate (unless dictated otherwise by solubility problems). For toxic compounds, only the highest dose level tested should show evidence of toxicity.

Inconclusive. When a test article cannot be identified clearly as a mutagen or nonmutagen in the standard plate assay, the results are classified as inconclusive.

References

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RESULTS AND DISCUSSION

3M Company's Compound T-3727 was screened for mutagenic activity in the Ames Salmonella/microsome in vitro mutagenicity assay using the five standard strains of Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, and TA100. The assays were performed in duplicate, both in the presence and absence of a rat-liver metabolic activation system. Three plates per dose level were tested. Acetone was used as the solvent.

The microbial mutagenicity testing of this sample was performed on 20 and 27 February 1985. Dose levels ranging from 10 to 5000 μg/plate were used for both assays (Tables 1 and 2). No dose-related increases in the number of histidine-independent revertants were observed in either assay. A black precipitate was noted at 5000 μg/plate; therefore, these plates were hand-counted.

We conclude that Compound T-3727 was reproducibly nonmutagenic when tested according to these procedures.

Table 1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3727

Experiment Date: 20 February 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538			TA98			TA100		
Negative Control																	
Acetone	-	50 µl	20	22	32	3	10	3	17	18	17	18	26	32	125	124	129
	+	50	17	8	8	11	7	6	28	25	21	41	38	33	130	129	149
Positive Controls																	
Sodium Azide	-	1 µg	690	661	752										589	574	567
9-Aminoacridine	-	50				520	662	787									
2-Nitrofluorene	-	5							1440	1244	1552	942	895	1144			
2-Anthramine	-	1							23	34	27	37	36	34	150	136	151
	+	1							182	177	164	187	160	240	437	463	428
	-	2.5	37	37	27	8	11	6									
	+	2.5	405	347	338	87	89	95									
Compound T-3727	-	10 µg	25	15	25	10	7	5	19	19	17	30	19	26	117	125	116
	-	50	30	21	26	9	6	5	14	13	17	29	23	38	111	145	115
	-	100	28	32	20	8	8	6	8	16	10	22	26	26	109	122	96
	-	500	23	21	24	8	7	8	14	10	12	23	13	23	137	122	108
	-	1000	28	16	29	5	7	7	13	16	17	29	17	19	123	127	111
	-	5000*	25	23	17	6	12	6	11	12	7	23	24	22	109	127	122
	+	10	13	15	18	15	9	9	21	25	22	53	28	31	106	153	116
	+	50	10	15	12	5	10	8	12	24	17	37	44	35	111	124	118
	+	100	16	16	12	6	13	13	25	27	23	25	29	23	120	130	115
	+	500	5	11	9	5	15	9	25	24	22	28	24	27	112	136	99
	+	1000	12	10	6	11	19	4	24	20	21	41	33	36	141	118	126
	+	5000*	12	17	8	5	9	11	21	17	19	31	20	43	146	120	125

*Precipitated at this dose level; hand-counted.

Table 2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

COMPOUND T-3727

Experiment Date: 27 February 1985

Compound	Metabolic Activation	Compound Added per Plate	Histidine Revertants per Plate														
			TA1535			TA1537			TA1538			TA98			TA100		
Negative Control																	
Acetone	–	50 µl	10	22	19	7	6	10	8	11	11	19	21	18	98	92	90
	+	50	8	15	7	16	6	8	8	18	19	18	26	26	103	91	89
Positive Controls																	
Sodium Azide	–	1 µg	464	492	477										500	549	544
9-Aminoacridine	–	50				111	293	346									
2-Nitrofluorene	–	5							1253	1240	1102	783	689	737			
2-Anthramine	–	1							8	15	22	40	21	22	128	125	129
	+	1							193	158	151	141	168	158	382	378	334
	–	2.5	24	21	20	8	10	6									
	+	2.5	215	212	220	68	72	68									
Compound T-3727	–	10 µg	21	26	16	3	3	7	16	16	20	23	19	20	122	97	96
	–	50	20	24	17	8	5	7	18	14	9	28	19	20	97	120	101
	–	100	23	14	22	6	3	8	11	9	12	21	19	15	115	84	88
	–	500	20	13	17	4	9	8	18	11	13	32	26	19	85	103	93
	–	1000	23	25	20	7	4	6	7	16	10	30	12	20	108	98	94
	–	5000*	19	13	22	7	4	9	10	8	4	30	23	21	82	92	109
	+	10	11	10	8	16	12	20	11	11	11	32	24	27	138	113	128
	+	50	10	8	8	11	8	6	15	15	9	26	34	19	130	79	132
	+	100	10	15	17	9	10	11	17	21	12	26	27	18	114	94	113
	+	500	12	11	14	6	12	9	16	9	15	24	25	21	115	103	108
	+	1000	6	8	7	7	12	13	16	20	12	18	16	26	124	114	114
	+	5000*	10	7	11	11	9	9	20	13	17	32	26	36	122	114	127

*Precipitated at this dose level; hand-counted.